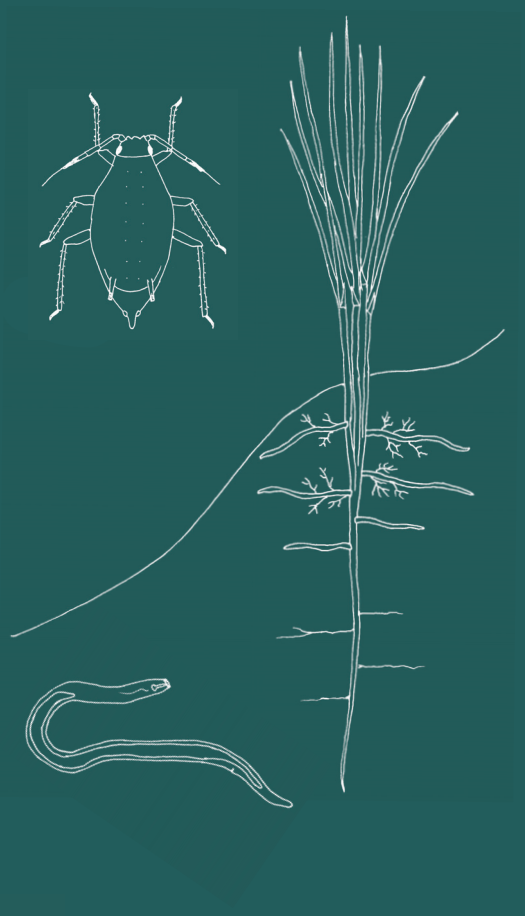
Environmental and genetic aspects of aboveground-belowground interactions in the *Ammophila arenaria* system

Martijn L. Vandegehuchte



UNIVERSITEIT GENT

Ghent University
Faculty of Sciences
Academic year 2010-2011

ENVIRONMENTAL AND GENETIC ASPECTS OF ABOVEGROUND-BELOWGROUND INTERACTIONS IN THE *AMMOPHILA ARENARIA* SYSTEM

OMGEVINGS- EN GENETISCHE ASPECTEN VAN BOVENGRONDS-ONDERGRONDSE INTERACTIES IN HET *AMMOPHILA ARENARIA* SYSTEEM

MARTIJN L. VANDEGEHUCHTE

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR (PHD) IN SCIENCES

PROEFSCHRIFT VOORGEDRAGEN TOT HET BEKOMEN VAN DE GRAAD VAN DOCTOR IN DE WETENSCHAPPEN

Promotor: Prof. Dr. Dries Bonte
Co-promotor: Dr. Eduardo de la Peña

Reading committee:

Prof. Dr. Wim Bert (Ghent University)

Dr. Martijn Bezemer (Netherlands Institute of Ecology)

Dr. Gerlinde B. de Deyn (Netherlands Institute of Ecology)

Prof. Dr. Hans Van Dyck (Université catholique de Louvain)

Other members of the examination committee:

Prof. Dr. Dries Bonte (Ghent University)

Dr. Eduardo de la Peña (Ghent University)

Prof. Dr. Dominique Adriaens (chairman, Ghent University)

Date of public defence: 17 December 2010

Vandegehuchte M. L. 2010. Environmental and genetic aspects of aboveground-belowground interactions in the *Ammophila arenaria* system. PhD Thesis, Ghent University, Ghent, Belgium.

ISBN: 978-90-9025921-5

The research reported in this thesis was conducted at the Terrestrial Ecology Unit (TEREC), Department of Biology, Faculty of Sciences, Ghent University. Address: Karel Lodewijk Ledeganckstraat 35, B-9000 Ghent, Belgium.

This research was funded by a predoctoral grant of the Research Foundation – Flanders (FWO).

Acknowledgements

This thesis is the product of four years of work, although in retrospect it seems much shorter. Maybe that is because it never really felt like 'work' to me. During this period many people helped or supported me in various ways. Without them, writing this book would not have been possible.

First of all, I would like to thank two people who were closely involved with my work: my supervisor Dries Bonte and co-supervisor Eduardo de la Peña. I am fully aware that not many PhD students have the sheer luck to be surrounded by such splendid guides, so a lot of credit for this thesis goes to them.

Dries was also the supervisor of my MSc thesis, and when he asked me whether I was interested in applying for a doctoral grant, I needed very little convincing. Dries, your unstoppable enthusiasm about science is downright infectious. In times when scientific excitement tends to dampen, you always reminded me of the bigger picture, the greater goal. Thanks also for your almost instantaneous answers to my numerous questions, many of those via e-mail at late hours and on Sundays, often about details of which the relevance was questionable. Eduardo, your sense of methodological detail often saved me a lot of practical disaster and lost time. Furthermore, your down-to-earth approach of science was often a good counterbalance in these times of publication pressure, where fancy concepts are sometimes valued higher than solid science. Your nice sense of absurd humour often provided a welcome distraction. Thanks also for kindly putting your office at my disposal to use as an aphid culture room, and your laptop to finish this book. However, and more importantly, I would like to thank both of you guys for encouraging me to develop my own ideas, for giving me the opportunity to be part of a constructive team, and last but not least for the fun times at conferences across the globe.

Jean-Pierre Maelfait started as the supervisor of my PhD. Unfortunately, he passed away before I had the chance to get to know him better. He was always prepared to give his well-thought-out ideas on any matter, and he

often saw that little detail that others had missed. His advice was much appreciated. He is and will continue to be missed at Terec.

Without Viki Vandomme and Hans Matheve, a lot of ideas that looked nice on paper would not have been put into practice. Thanks for driving me around, measuring grasses, digging up sand, carrying sand through the dunes and so on. In this context I would also like to thank the other lab members who, completely voluntarily, helped me out with my much dreaded field work. But I'll get to you guys in a moment. Viki, thanks for arranging all things administrative for me. This was a luxury that cannot be understated. I would also like to thank Thomas Terrie for the field assistance and the good gardening skills that kept my plants healthy and fresh.

I have been fortunate to have had two very good MSc students during my PhD, Bart Vandenbossche and Roeland Cortois. Bart, thanks for the detailed work on the nematodes and good luck with the research in Germany. Roeland, thanks for delivering such good work, despite the fact that I unintentionally sabotaged your plant stock once. I hope all goes well with the PhD in the Netherlands.

I am furthermore grateful to Dr. Peter Breyne for performing the AFLP analyses of the grasses.

I would like to thank Dr. Oscar Vorst for checking beetle identifications, Dr. Roland Mühlethaler for checking leafhopper identities and Prof. Dr. Juan Nieto Nafría and Dr. Nicolás Pérez Hidalgo for confirmation of aphid identifications.

I am also grateful to the members of the reading committee for their valuable comments and suggestions on the previous version of this thesis.

To the guys of the lab, Boffie, Bram(bo), Bram (b-dog), Stevo, Hans, Charlie and the others, thanks for Brabor-Hanmar, the food-bot, the whisky tasting, the pub nights, the parties, the coffee table discussions that had to be settled by Wikipedia, the field weeks at the coast in June and the numerous other joyous events that I can't think of right now. Thanks also for getting me from behind my desk for some sports every now and then. My levels of darts and pool would not have been the same without you guys.

Thanks also to Kevin for the practical jokes which on regular occasions cheered up everyone but the victim, and thanks to the block of lead for not killing anyone in the process.

I would like to thank Carl Vangestel, who was always willing to put aside more urgent matters in order to answer my statistical questions. Carl, the way you manage to explain something abstract in a few lines and words is admirable.

The many conferences and workshops that I have attended over the past four years would have been much duller without their social evening aspect. I don't know how we did it, but we always seemed to quickly find those people who valued this social aspect as much as we did. It was people like Yann Hautier, Klaus Birkhofer, Stefanie Kortman and Sarah Kenyon, to name just a few, who made the difference. If it weren't for conferences, I would probably never have met Lucía Latorre Piñeiro. Lu, thanks for the hospitality and for guiding us around in Mallorca as well as in Galicia, and for the good times we had both in Spain and back here in Belgium. You're the living proof that scientists needn't be boring. Good luck with finishing the PhD and with the band. And by the way, say hi to your mom.

I would like to thank my parents, commonly referred to as Matje and Patje, who have always encouraged me to pursue my own interests. I also want to thank my two brothers. Michel, thanks for so often paving the way for me (even though you might have put the standards a bit high). Mau, within a few years it's your turn, and I'm sure you will do a great job, which you usually do with everything you undertake. Then the world will know three doctors M. Vandegehuchte, which might come in handy when making publication lists. Thank you guys for always being there.

Furthermore, I would like to thank my grandfather, Louis Ven, a man with a keen interest in science. He encouraged me throughout my higher education and was always eager to know what I was working on.

Thanks to Brecht, Thijs and Karl for keeping the comradeship alive. I think of the ski trekking in Lapland and Jämtland, the Natuurpunt quizzes and the countless gatherings in pubs throughout Ghent. I know many of the crazy plans for journeys to desolate places ended up in the drawer because of the

PhD, but let's take them out and see what can be done. Dave, thanks for those entertaining evenings with their sumptuous dinners, potent coffee and South Park episodes. May many of those follow.

Bieke, you never made a fuss when, yet again, work got the highest priority. Thanks for your patience, support and optimism. Thanks also for all the good times we had over the past years, including our travels and small trips. Moreover, thanks for proofreading the entire thesis. Life just seems so much easier with you. Bie, thanks for being there and being who you are.

Again to all of you people: thanks!

Martijn L. Vandegehuchte

6 December 2010

Table of contents

1 Introduction

Martijn L. Vandegehuchte

1.1 The emerging awareness of the importance of aboveground-belowground interactions

For a long time, the bulk of ecological theory has been based on the findings of aquatic and aboveground ecologists, the vast soil biology literature notwithstanding. Studies on belowground organisms still make up just a small minority of articles in the major ecological journals (Wardle 2002). This is a surprising discrepancy, especially given the overwhelming diversity of the biota living in soils. The most obvious practical impediment to the development of an ecology of soils, is the sheer difficulty of observing soil organisms in situ. Therefore, aboveground ecologists, although generally acknowledging the importance of belowground organisms, have been furthering ecological knowledge and concepts largely independent of soil biologists. The latter, on the other hand, have traditionally ignored to a large extent what went on above the soil surface.

A few decades ago, however, interactions that take place between the above- and belowground components of ecosystems started to spark the interest of ecologists. In recent years, a diversification has taken place of the specific research questions addressed in the context of aboveground-belowground ecology, leading to several subdisciplines in this field. Results of these studies have created a growing awareness that aboveground-belowground interactions hold consequences for community organisation and ecosystem processes.

The earlier work focused mainly on plant-soil feedbacks and the importance of soil biota in determining plant nutrient acquisition, growth and community structure (Wardle 2002), and more specifically on the role of soil biota in plant succession. A next step was the investigation of links between above- and belowground plant consumers. The realisation soon followed that these interactions could further cascade through the higher trophic levels, at either side of the surface, placing aboveground-belowground ecology in a multitrophic context. Further research has focused on interactions including plant mutualists, such as mycorrhizal fungi. Some of the general ecological issues to which aboveground-belowground interactions have recently found their way are the management of agro-

ecosystems, global change and invasive species biology. Several of these specific areas of aboveground-belowground ecology have been reviewed, such as the interactions between belowground decomposers and aboveground invertebrate (Scheu 2001) and vertebrate herbivores (Bardgett and Wardle 2003), aboveground and belowground insect herbivores (Masters et al. 1993), mycorrhizal fungi and insect herbivores (Koricheva et al. 2009) and aboveground-belowground multitrophic interactions (van der Putten et al. 2001).

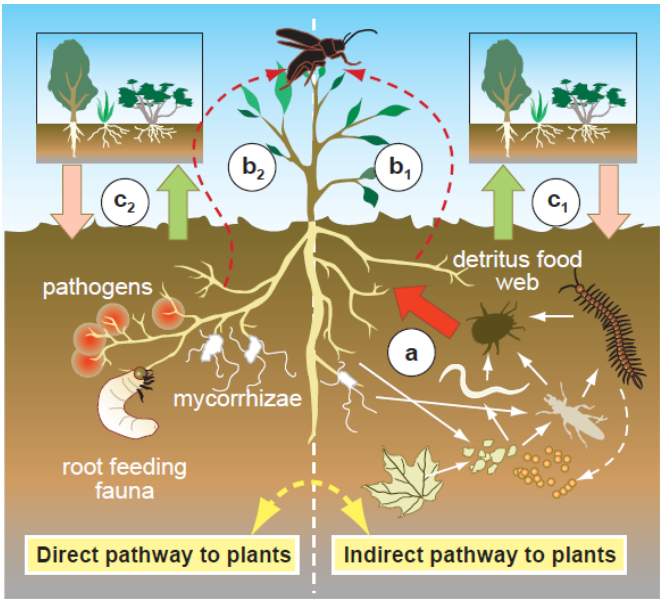


Figure 1.1 Overview of the direct and indirect effects of soil organisms on plants an aboveground fauna. Right panel: feeding activities in the detritus food web (white arrows) enhance nutrient turnover (thick red arrow), plant nutrient acquisition and performance (a) and thus indirectly affect aboveground herbivores (red broken arrow) (b_1). Left panel: soil biota exert direct effects on plants by feeding on or infecting their roots or forming a mutualistic relationship with the host plant. These direct effects on the host plant further affect the performance of shoot herbivores (b_2) and potentially their predators. Further, the soil food web can control the successional development of plant communities both directly (c_2) and indirectly (c_1), and these plant community changes can in turn feed back to the soil biota. Source: Wardle et al. 2004. Science 304: 1629-1633.

In general, two approaches are taken to aboveground-belowground interactions research (van der Putten et al. 2009). The so-called primary

producer approach considers how plants, root and shoot dwelling herbivores, pathogens and symbionts and their predators and possible predators of these predators interact through various direct and indirect pathways. The detritus approach, on the other hand, assesses how dead organic material, microbial decomposers, detritivores, microbivores and predators interact (see Fig. 1.1). The decomposition of detritus greatly affects the availability of nutrients to plants, which in turn has consequences for the different components of both the above- and belowground food web (Wardle 2002). Note that both approaches are not necessarily mutually exclusive, and mixed concepts have been proposed (Scheu 2001, Wardle et al. 2004). As explained below (see 1.7), this thesis only focuses on the primary producer approach. Therefore, I will not go further into the role of detritus in aboveground-belowground interactions here. To some extent relating to the detritus approach, is the large body of literature on the important and often complex effects of vertebrate herbivores, especially mammals, on root exudation, root biomass, leaf nutrient concentration, litter quality and return of nutrients to the soil through dung and urine, all of which can exert effects on the soil community. Excellent information on this topic can be found in Bardgett and Wardle (2003) and Wardle (2002).

Although it is clear that the study of aboveground-belowground interactions has become a fast-growing area of ecology, several aspects of these interactions still remain largely unexplored. For example, although widely acknowledged as potentially important, the role of the abiotic soil component in modifying these interactions between above- and belowground biota has hardly been investigated formally. Another observation is that research on aboveground-belowground interactions still seems to be largely restricted to a purely ecological context. The consequence of interactions that cross the boundary between soil and air for the evolution of the organisms that take part in these interactions, as well as the other organisms in the community, still remains an open question (but see Bonte et al. 2010, de la Peña et al. 2010). Moreover, the research on aboveground-belowground ecological linkages is still mainly conducted by means of controlled laboratory experiments using a small number of interacting species. Such experiments are very useful to uncover the causality of aboveground-belowground links as well as to obtain an idea about the generality of their directions and strengths. However, whether

conclusions about these aboveground-belowground interactions still hold, in a realistic context of multiple species and abiotic factors at both sides of the surface, can only be revealed by field observations and experimentation. In this PhD thesis, an attempt is made to answer some of these yet unresolved issues.

1.2 The primary producer approach

Because of their unique property of possessing both above- and belowground organs, plants enable different types of interactions and feedbacks between the above- and belowground community. These interactions can be mediated by changes in the living plant, or take place through changes in the amount and quality of leaf litter entering the soil (Wardle et al. 2004). Changes in the living plant broadly fall into two categories. The first comprises all changes that lead to directly or indirectly altered availability of resources to organisms at the other side of the soil surface. This includes changes in limiting nutrient or water content of the plant's tissues, available plant biomass, plant morphology and architecture. The second category consists of changes in the plant's defences, which can be further divided into direct and indirect defences. Direct defences can be defined as plant structures, such as trichomes, or chemical compounds, such as toxins, deterrents or digestibility reducers, that directly reduce the preference or performance of a herbivore or pathogen (Bezemer and Van Dam 2005). Indirect defences, on the other hand, are means by which the plant recruits carnivores or enhances their efficiency in attacking herbivores (Bezemer and Van Dam 2005). These include plant-produced compounds for attraction, such as volatiles, additional food sources such as extra-floral nectar and plant structures that provide shelter to carnivores, for example domatia (Bezemer and Van Dam 2005). Plant defences can furthermore be divided into constitutive defences, which are always expressed, and induced defences, which are only activated following herbivore attack or pathogen infestation (Bezemer and Van Dam 2005). Interactions between above- and belowground organisms are possible through changes in each of these plant traits. Often the exact mechanism is highly complex, because nutrients and defence are typically interlinked. The production of both chemical and

morphological defences depends on nutrient availability (van der Putten et al. 2001).

Apart from plant consumers, also predators and parasitoids have been shown to be affected by organisms on the other side of the soil surface. Since the focus of my research is mostly on the interactions between root and shoot herbivores as well as between shoot herbivores and root mutualists, those higher level effects will not be further discussed. More details on these multitrophic aboveground-belowground effects can be found in Bezemer et al. (2005), González-Megías and Müller (2010), Guerrieri et al. (2004), Neveu et al. (2002), Soler et al. (2005), Soler et al. (2007a), Soler et al. (2007b), Soler et al. (2007c) and van der Putten et al. (2001).

1.3 Herbivore-herbivore interactions

Interactions between above- and belowground herbivores have first been brought to the attention by the work of Gange and Brown (1989) and Moran and Whitham (1990). This early work inspired the seminal paper by Masters et al. (1993), which describes a conceptual model of the plant-mediated interactions between root- and shoot-feeding insect herbivores (see Fig. 1.2). This model assumes that by removing fine roots, root herbivores induce a stress response within the host plant that is associated with the accumulation of soluble amino acids and carbohydrates in the foliage, which increases the performance of foliar-feeding insects. On the other hand, foliar herbivory is expected to cause a decrease in root biomass, and because roots are a poor quality food resource, any reduction in root quantity should have a negative effect on the root-feeder. This plus-minus interaction model was based on the earlier observations that plant stress can lead to increased insect performance (White 1969, White 1984), and on a handful of studies investigating the effect of root on shoot herbivores and/or vice versa (Gange and Brown 1989, Masters et al. 1993, Moran and Whitham 1990, Seastedt and Reddy 1991). Interestingly, Masters et al. (1993) already list some exceptions to their conceptual model (Seastedt et al. 1988), and put forward a more complex interaction involving mycorrhizal fungi as an explanation (see 1.4).

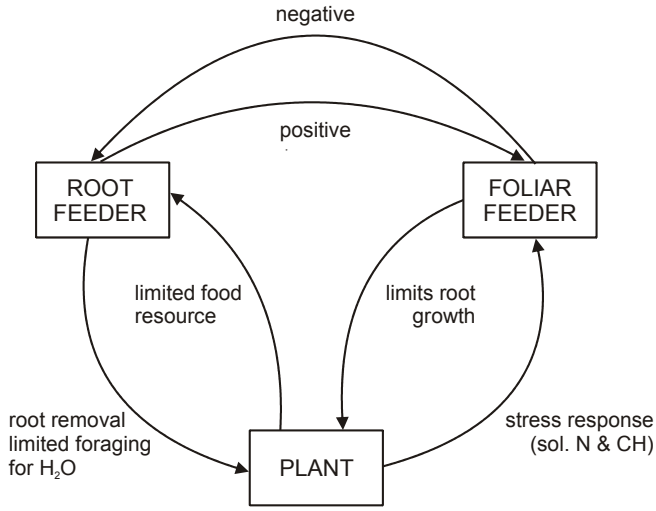


Figure 1.2 Conceptual model of the plant-mediated interactions between root- and shoot-feeding insect herbivores. Adapted from Masters et al. 1993. Oikos 66: 148-151.

Since these first steps towards a conceptual understanding of the mutual interactions between root- and foliar-based herbivores, studies on this topic have proliferated. It has become increasingly clear that the model of Masters et al. (1993) is not as generally valid as originally thought. Positive, negative and neutral effects between above- and belowground herbivores in either direction have been demonstrated (Blossey and Hunt-Joshi 2003, De Deyn et al. 2007, Masters 1995, Masters et al. 2001, Salt et al. 1996, Staley et al. 2007, Staley et al. 2008, Tindall and Stout 2001), and the mechanisms by which these interactions operate are equally diverse. Negative effects could often be ascribed to the induction of chemical defences by herbivory in one part of the plant that spread to other plant parts and thus affected herbivores feeding on the other side of the soil surface (Bezemer et al. 2003, Bezemer and Van Dam 2005, Soler et al. 2007a, van Dam et al. 2003). The original emphasis on plant nutrients as mechanisms of interactions between root and shoot herbivores therefore has shifted towards investigating defensive compounds as the principal mechanism. However, Johnson et al. (2009) demonstrated complex effects of shoot-feeding aphids on both mineral nutrient and amino acid content and composition, which could be modified by the presence of wireworms (larval Elateridae). Moreover, aphids caused a decrease in most leaf minerals, but an increase in most root

minerals, which probably caused the positive effect on wireworms. Another example of the complexity of these interactions is provided by Kaplan et al. (2008), who could demonstrate mutualistic interactions between root-feeding nematodes and aboveground phytophagous insects on tobacco plants. The positive effect of nematodes on leaf feeders could be attributed to the destruction of production sites of nicotine in the roots, which led to lowered nicotine concentrations in the leaves. The positive effect of the foliar herbivores on the root feeders was caused by a reallocation of foliar resources to roots. In contrast to the early conceptual model, Bezemer et al. (2005) found that nematodes caused a lowering of amino acid levels in the leaves of a grass, which negatively affected aphid performance. These examples should suffice to highlight the overwhelming diversity and complexity of mechanisms capable of causing root and shoot feeders to affect each other through changes in their mutual host-plant.

Although some studies, including some of the early ones, have made efforts to explore the interactions between root and foliar herbivores in the field, most of them still concern either controlled laboratory experiments or field manipulations, often in quite unnatural circumstances, using agricultural crop species. The great merit of these studies is that they uncovered the existence and diversity of these aboveground-belowground herbivore interactions. However, if we truly want to understand the importance of these interactions in the natural world, where numerous other biotic and abiotic variables act on the performance of both plants and herbivores, a next step is to investigate whether patterns in nature correspond to predictions based on the results of these experiments.

1.4 Herbivore-mutualist interactions

Although interactions between pollinators, which are aboveground mutualists, and root feeders have been demonstrated (Poveda et al. 2005), our model plant species is wind-pollinated (see 1.7). Therefore, this section focuses on interactions involving mutualistic fungi. Mycorrhizal associations between a fungus and a plant root are ubiquitous in the terrestrial environment. Seven types of these associations can be distinguished (i.e. arbuscular, ectomycorrhiza, ectendomycorrhiza, ericoid, arbutoid, orchid and

monotropoid) based on the type of fungus and the different structures produced by the root-fungus combination (Smith and Read 1997). A range of functions of mycorrhizal symbiosis have been described, including defence, nutrient uptake, soil aggregation stability and drought resistance (Hodge 2000). Of the seven types, the most common association is the arbuscular mycorrhiza (AM), which is formed by approximately 70% of the world's vascular plants (Brundrett 1991, Hodge 2000). Since our study system is characterised by the presence of this type of mycorrhiza, I will further focus on the arbuscular mycorrhizal fungi (AMF). These are obligate symbionts that establish a symbiosis with a plant from which they obtain carbon. In exchange, they assist the plant with the acquisition of mineral nutrients, particularly phosphorus (Harrison 2005). They are also known to improve the drought resistance of plants, often indirectly via improved nutrient acquisition which in turn leads to growth enhancement, but also through several direct hydraulic effects (Augé 2001). In most cases AMF have thus been shown to act as mutualists to the plant. However, the effects of these mycorrhizae on plant performance can also be neutral or become negative under circumstances where net costs exceed net benefits (Graham and Abbott 2000, Koide 1985, Reynolds et al. 2006, Sudova and Vosatka 2008). In a theoretical model, Gange and Ayres (1999) propose a curvilinear relationship between plant benefit and percentage of roots colonised by AMF, so that beyond a certain density, AMF become antagonists to the plant. The shape of this curve, and hence the density of AMF at which plant benefit is maximal, might differ according to plant and fungus species identity, soil biotic or abiotic conditions, or level of irradiant light (Hoeksema et al. 2010, Johnson et al. 1997). Especially when the AMF-provided limiting soil resource is increased, and root colonisation by AMF does not decrease, the mutualistic interaction often changes into a parasitic one (Hoeksema et al. 2010, Johnson et al. 1997). In the first weeks following germination, plants obtain their necessary resources from seed reserves. In this stage, AMF can become parasitic, because the loss of carbon to the fungus decreases allocation to photosynthesis or defence. However, these short-term losses are often compensated by nutrient gains in the long term (Johnson et al. 1997). Nitrogen-limited plants are moreover expected to gain relatively less benefit from associations with AMF than phosphorus-limited plants, since nitrogen limitation reduces plant photosynthetic capacity and hence the availability of carbon for the mycorrhiza (Hoeksema et al. 2010). Therefore, benefit is

predicted to be greatest for phosphorus-limited plants that are, however, not nitrogen-limited.

Given the diversity of physiological responses that mycorrhizal fungi can induce in plants, effects on aboveground herbivores seem almost self-evident. Two recent studies on the effects of mycorrhizal fungi on insect herbivores, a review (Hartley and Gange 2009) and a meta-analysis (Koricheva et al. 2009), revealed that strengths and directions of effects varied greatly. A general trend however emerges when accounting for insect feeding guild and host specificity. Generalist chewing insects and mesophyll feeders appear to be mostly negatively affected by the presence of mycorrhizal fungi, whereas all phloem feeders and specialist chewers are usually benefited (Hartley and Gange 2009, Koricheva et al. 2009). This observation lends support to the notion that mycorrhizal effects on insect herbivores are primarily due to the activation of plant defences. Chewing and sucking insects feeding on cell contents are indeed more prone to ingestion of secondary metabolites, which are commonly stored inside cells, than sucking insects feeding on phloem, which is largely void of any defensive compounds (Koricheva et al. 2009). Nevertheless, some studies have demonstrated effects of AMF on insect herbivores through altered plant nutrient status (Gange and Nice 1997, Gange et al. 2005, Goverde et al. 2000). Myccorhizal fungi were observed to lower the N content of *Cirsium arvense* thistles and to reduce the growth of *Urophora cardui* gall flies. However, this effect of mycorrhizae could be counteracted by the addition of N (Gange and Nice 1997). The authors propose that the underlying mechanism was a delay in the appearance of secondary nutritive tissue in the gall, caused by a lowering of host plant N or a modification of the form of plant N. Carbon-based defences were dismissed as an explanation, because the C:N ratio of gall tissue was unchanged and because of the fact that these defences are known to have little impact on galling insect performance. The drastically improved performance of *Polyommatus icarus* caterpillars fed with sprigs of mycorrhizal *Lotus corniculatus* (Goverde et al. 2000) was also attributed to changes in plant quality, more specifically the threefold increase in the content of P, a known determinant of insect growth. However, mycorrhizal plants also had a higher C concentration, which relates to sugar composition, phagostimulants as well as allelochemicals. Gange et al. (2005) found positive effects of mycorrhizal fungi on the performance of

leaf-mining and seed-feeding flies on *Leucanthemum vulgare* in the field. These effects were dependent on the specific mycorrhizal species or combination of species and of the time of the year. Enhancement of leaf-miner performance was attributed to increased N content of the plants, while increased attack by seed feeders was linked to the increased number and size of flowers. These examples demonstrate that plant defence is probably not the only mechanism causing interactions between mycorrhizal fungi and aboveground herbivores. However, it remains hard to unambiguously demonstrate nutrient-mediated mechanisms, because the concentration and composition of nutrients and allelochemicals are often interlinked. Moreover, substances that act as allelochemicals to one species of herbivore might act as phagostimulants to others (Schoonhoven et al. 2005). In addition, experiments assessing the impact of mycorrhizal status of the host plant on herbivore performance using individual leaves or leaf discs might fail to detect effects of changes in overall biomass that can be important in the field. This is suggested by Goverde et al. (2000), who observed a 33 times higher total biomass of mycorrhizal plants, but only used detached leaves in their assessment of effects on the herbivore.

1.5 The interplay with abiotic soil factors

From the previous paragraphs it has become clear that plant-mediated interactions between above- and belowground biota have been extensively investigated. This research has demonstrated the bewildering diversity of possible interactions between above- and belowground organisms. However, the effect of the abiotic component of soils on these interactions has rarely been considered. This is at least surprising, given the obvious importance of abiotic soil factors such as mineral nutrient and water availability to the performance of plants. It therefore seems highly plausible that changes in abiotic soil characteristics are able to alter the interactions between soil organisms and their aboveground counterparts.

Because of the long-appreciated role of nutrient availability in the outcome of mycorrhizal fungi-plant associations (see 1.4), the research on interactions between mycorrhizal fungi and herbivores forms a notable exception. In their meta-analysis Koricheva et al. (2009) found that

manipulating levels of N alone did not modify responses of chewers or sucking insects to mycorrhizae. Manipulating levels of P significantly affected responses of sucking insects, which where greater at low P levels, but not those of chewers (see for example Borowicz 1997, Gange et al. 1999). Koricheva et al. (2009) furthermore suggest to include other factors such as sterols, essential amino acids, proteins, carbohydrates, vitamins or fatty acids into studies of insect-mycorrhizae interactions, as all of these can be limiting for certain insects. For example, aphids are known to use sucrose concentration gradients created by diffusion of sucrose out of phloem elements to locate their food source. Moreover, AMF are able to increase leaf sucrose concentrations as well as the size of the vascular bundles. It is therefore possible that phloem-feeding aphids generally respond positively to mycorrhizal presence, because the degree of success in locating phloem is higher on mycorrhizal plants. This very interesting hypothesis currently remains to be tested.

Apart from those involving mycorrhizal fungi, only few studies investigating aboveground-belowground interactions have considered the effects of the abiotic soil component. Two studies by Staley et al. (2007, 2008) have assessed the interactions between above- and belowground insect herbivores under different levels of water availability. In their first study, Staley et al. (2007) found that the negative effect of root-chewing *Agriotes* larvae on leaf-mining *Stephensia brunnichella* larvae reared on *Clinopodium vulgare* disappeared when plants were subjected to a severe drought treatment. In their second study (Staley et al. 2008), the interaction between the larvae of *Agriotes lineatus* and the leaf-mining *Chromatomyia syngenesiae* on four different *Sonchus* species were investigated. Interestingly, in this study a positive effect of the root-feeder on the performance of the leaf-miner was detected, albeit on just one of the host plant species. Moreover, this interaction only occurred when plants were subjected to drought. These results indicate that water availability can be important in the outcome of interactions between root and shoot herbivores, and that these interactive effects can differ according to the species involved. Haase et al. (2008) investigated the interactions between decomposers and leaf herbivores, namely collembolans (*Folsomia candida*) and aphids (*Rhopalosiphum padi*), on the grass *Poa annua* under different levels of nutrient availability. Collembolans strongly increased aphid numbers

at low and moderate nutrient availability, while this effect was much weaker at high nutrient availability. This effect was attributed to the increased quantity or quality of nitrogen-based compounds. In turn, aphid herbivory increased the number of Collembola only at high nutrient availability, which was hypothesised to be due to changes in root exudation and carbon allocation or a stimulation of microbial biomass through honeydew excretion.

Although these cited studies on aboveground-belowground interactions have considered differential nutrient availability to the host plant, this was usually realised by applying low or high levels of fertiliser, often changing only one or a few specific elements. The amount of nutrients that is administered often differed by an order of magnitude. Moreover, the densities of soil organisms applied in these studies were not always representative of field conditions, and most often only one species was added to the soils. The question thus remains whether findings from such experiments are informative of the interactions between abiotic and biotic soil components in their effect on aboveground organisms in the field, where soil biota comprise a multitude of interacting species and where the abiotic status of soils is determined by many different variables. A convenient methodology in this respect is provided by Joosten et al. (2009), who inoculated two different types of sterilised soil, calcareous sand from a coastal dune area and a mixture of sand and potting soil from an experimental garden, with 5% of non-sterile soil of each soil type. The organisms present in the inoculum were given time to proliferate in the bulk soils. On these soils they grew two genotypes of *Jacobaea vulgaris*, differing in pyrrolizidine alkaloids (PA). This setup allows to decouple the effects of the abiotic (sterile soil) and biotic (inoculum type) soil components on different plant characteristics. The sterile soil origin had a greater impact on plant dry mass than the inoculum. However, plant dry mass was highest on sterile soils and lowest on sterile soils inoculated with soil of the same origin. Both the abiotic and biotic soil component, moreover, affected the PA composition of the plants, including the concentrations of specific PAs known to be toxic to generalist herbivores but attractive to specialists. Unfortunately, Joosten et al. (2009) did not test this potential effect on actual herbivores. The main disadvantage of this approach is that the soil community remains a black box. However, its merit is in the use of field soils, which makes it possible to

assess the relative importance of the biotic and abiotic aspect of soil to the performance of plants and their herbivores under natural circumstances, including the interactions between the different types of organisms and the different compounds present in soil.

1.6 Evolutionary consequences of aboveground-belowground interactions

Although the ever-increasing research effort has greatly enhanced our understanding of the ecology of aboveground-belowground interactions, little is currently known about the evolutionary implications of these interactions. The evolution of plants in response to herbivores and vice versa have been the subject of many studies (Futuyma 2005, Schoonhoven et al. 2005). However, most of these studies were restricted to the investigation of aboveground herbivores. Based on theoretical models, the effect of the soil community on the evolution of plants might be substantial (Bever et al. 1997). Evidence that soil organisms with known effects on plant fitness, such as certain root-feeding nematodes, select on different genotypes of species is provided in the agricultural literature (Fuller et al. 2008, van der Putten et al. 2006). Many crop species indeed consist of different cultivars with different susceptibilities to root feeders. It therefore seems highly plausible that the soil food web is important for the evolution of plants. Because of the numerous types of plant-mediated interactions between above- and belowground organisms that have been demonstrated over the past years, the importance of these interactions is by now well-acknowledged in community ecology. Paradoxically, the consequences of these interactions for evolutionary dynamics have hardly been addressed (Barot et al. 2007). The question thus arises whether the influence of soil organisms on the evolution of plants further affects the evolution of aboveground animals associated with these plans, or in the opposite direction, whether selection pressures exerted by herbivores on plants affect the evolution of belowground organisms. One study demonstrates that it is possible for belowground organisms to affect the evolution of aboveground herbivores (Bonte et al. 2010). In a laboratory experiment, *Tetranychus urticae* spider mites were bred for 15 generations on *Phaseolus vulgaris* grown on sterile

soil, soil inoculated with root-feeding *Pratylenchus penetrans* nematodes or soil inoculated with *Glomus mosseae* AMF. Subsequently, the performance of mites from each of the selection lines was investigated on plants subjected to each of the soil treatments. When several life history traits were integrated into one fitness measure, the performance of the mites was consistently better on the plant ecotype on which they had been bred. This demonstrates that soil organisms were indeed able to impose selection on the aboveground herbivores, mediated by the host plant. Such results provide a hopeful first step towards the appreciation of aboveground-belowground linkages in evolutionary processes. However, in natural ecosystems, plants are faced with a multitude of interacting above- and belowground organisms.

A major question in evolutionary ecology is how coevolution operates in a multiple species community context, or in other words, how the evolutionary interactions between two species are affected by the presence of other species in the community (de la Peña et al. 2010). The prevalence of multispecies interactions has led to the distinction between two types of coevolution: pairwise and diffuse. In its strictest definition, pairwise coevolution implies that the coevolutionary dynamics of each pair of species can be understood without considering any other species (Janzen 1980). Diffuse coevolution implies that interactions with additional species have to be taken into account to understand the coevolutionary dynamics between a focal pair of species (Janzen 1980). This issue has been mostly investigated in the context of the coevolution between plants and their natural enemies. For this coevolution to be pairwise, several conditions have to be met. Firstly, there are no genetic correlations between a plant's resistance to the focal herbivore and resistances to auxiliary herbivores. Secondly, selection on resistance to any natural enemy does not depend on the presence or absence of other natural enemies (Hougen-Eitzman and Rausher 1994, Iwao and Rausher 1997). If either of these conditions is not met, coevolution is diffuse. Two non-exclusive mechanisms can lead to the violation of this second criterion (Hougen-Eitzman and Rausher 1994). Firstly, the presence of auxiliary herbivores could alter the amount of damage caused by the focal herbivore in a way that alters the pattern of selection. Secondly, the fitness consequences of focal herbivore damage could depend on the presence of auxiliary herbivores. Furthermore, if there is an absolute genetic correlation

between the resistance to the focal herbivore in the absence and that in the presence of auxiliary herbivores, the same loci contribute to resistance in each case, and coevolution is pairwise. However, if this correlation significantly differs from 1, at least some loci that confer resistance to the focal herbivore differ in the absence and presence of other herbivores, and coevolution is diffuse (Stinchcombe and Rausher 2001). Only a few studies on plants and their aboveground enemies have investigated all these criteria to distinguish between pairwise and diffuse coevolution. However, the existence of genetic correlations between the susceptibilities of plant genotypes to different herbivore species has been the subject of several studies, probably because this is the easiest of the criteria to test. In a review of these studies, Leimu and Koricheva (2006) found that in general, genetic correlations between resistances to multiple natural enemies were positive, suggesting a prevalence of diffuse coevolution and generalised defences. However, the large variation in the strength and direction of the associations suggested a continuum between pairwise and diffuse selection. None of the 29 included studies of 16 different plant species included correlations involving belowground organisms. Therefore, our current understanding of the coevolution of plants and their enemies seems entirely based on only half of the community that is associated with a plant.

Recent studies show that genetic variation in a single species can have predictable effects on community structure and ecosystem processes. Because these 'community and ecosystem phenotypes' have a genetic basis and are heritable, researchers are beginning to apply the principles of population and quantitative genetics to construct an evolutionary framework for complex communities and ecosystems. An update of advances in this field and a review of plant species whose genetics influence the associated communities are provided by Whitham et al. (2006). Currently, the belowground community has hardly received attention in this context. The best-studied case in community and ecosystem genetics concerns the tree species *Populus angustifolia* and *Populus fremontii* who naturally hybridise in a small region in Utah. In these trees, genetically determined levels of condensed tannins affected arthropods living in tree canopies, endophytic fungi in the bark, as well as aquatic macroinvertebrates feeding on leaves after they have fallen into a stream. Condensed tannins moreover explained the majority of variation in nitrogen mineralisation in the soil and

decomposition of leaves in the stream (Whitham et al. 2006). Schweitzer et al. (2008) found that the composition of microbial soil communities associated with these *Populus* trees could also be explained by genotype. This result demonstrates that extended phenotypes are not restricted to aboveground communities. However, studies addressing the responses of multiple above- and belowground organisms to plant genotypes are still lacking. A first step towards incorporating aboveground-belowground ecology into the emerging field of community and ecosystem genetics, would be to investigate whether plant genotypes affect the associations with both above- and belowground members of the associated community. The investigation of correlations of pairs of above- and belowground species across these genotypes would furthermore be a first prerequisite to a more holistic understanding of the process of coevolution.

1.7 *Ammophila arenaria*: a multifunctional study system

To address some of the issues mentioned earlier, I chose *Ammophila arenaria* (L.) Link as a model system. This is a perennial, rhizomatous grass that naturally occurs along all European coasts south of latitude 63° N. Two subspecies occur within this range, ssp. *arenaria* occurs in the north of the range while ssp. *arundinacea* is found from Portugal southwards along the Mediterranean and Black Sea coasts (Huiskes 1979). This division was originally based on differences in leaf, panicle and flower morphology. In *A. arenaria* ssp. *arundinacea* the hairs surrounding the base of the lemma are half the length of the lemma, while in *A. arenaria* ssp. *arenaria* their length is one third of that of the lemma. *A. arenaria* grows most vigorously in the open habitat of mobile sand dunes (Huiskes 1979). In these early successional sand dunes, *A. arenaria* is the dominant species, often occurring as monospecific stands (Huiskes 1979). Clonal spread is the main mode of reproduction in the field, as almost all seedlings die due to desiccation, burial or small-scale sand erosion. Only in areas where the soil surface stays moist, such as young slacks, the risk of desiccation or small-scale erosion is small and seedlings can establish (Huiskes 1979). As soon as sand accretion ceases and dunes start to become stabilised, *A. arenaria* starts to decline, becoming

less robust, with shorter leaves, fewer inflorescences and a decreased shoot weight per plant (Eldred and Maun 1982, Huiskes 1979). Therefore, vigorous stands of *Ammophila* are found in foredunes and large, dynamic dune areas with drifting sands, while degenerate relics can be found in stabilised dunes, often at the inner dune edge. Thus, not only does *A. arenaria* tolerate sand burial up to 1 m per year (Huiskes 1979), this species actually needs substantial burial to remain vital.

This loss of vigour under decreased sand dynamics has been known among botanists for a long time, and was coined 'the *Ammophila* problem' by Marshall (1965). The same phenomenon is also observed in the morphologically very similar North American congener *A. breviligulata*. Several hypotheses have been put forward to explain the *Ammophila* problem, some of them dating back more than a century. Buchenau (1888) attributed the decline in vigour of *Ammophila* in stabilised dunes to the increase in organic material. Other early explanations, including a lack of nutrients in the absence of wind-blown sand, an age effect, poor aeration of roots and rhizomes, presence of toxic secretion substances, increased competition from other plants and increased soil acidity, were assessed by Halwagy (1953). He dismissed all of them except competition with other plants. He concluded that vigorous growth resulted from the effective reduction or elimination of competition by sand burial in addition to the increase in moisture content of the sand. Based on experiments with *Corynephorus canescens*, Marshall (1965) proposed that competition by roots of other species in sand layers where the site of adventitious root production is located, leads to a lowered replacement of old roots by new adventitious roots, which is further impaired by the drying out of the upper sand layers during summer. The elimination of this competition by burial and the additional volume of moist sand would thus enable the maximal development of new roots necessary for vigorous growth. Based on nutrient addition experiments, Willis (1965) agreed with this hypothesis, stating that continuous root development is probably necessary because large volumes of sand have to be exploited in order to obtain enough nutrients. Huiskes (1977) opposed this conclusion, and hypothesised that it is the tolerance to sand burial that gives *A. arenaria* the competitive advantage in dynamic dunes. Also for the North American *A. breviligulata*, competition with other plant species has been proposed as an explanation of the *Ammophila*

problem (Eldred and Maun 1982). Wallén (1980) proposed that the cessation of sand deposition initiates an ageing process in the plant, although the observation that degenerating plants can be rejuvenated by the addition of sand or fertiliser (Marshall 1965, Willis 1965) renders this mechanism unlikely. Other studies have shown that sand burial increased the net photosynthetic rate (Yuan et al. 1993) and stimulated the formation of new stem nodes, a prerequisite for shoot elongation (Voesenek et al. 1998), in *A. breviligulata*.

One of the first studies proposing a role for soil-borne organisms in the *Ammophila* problem, was that by Nicolson (1960) who linked the ecology of this grass to the presence of vesicular arbuscular mycorrhizae, and demonstrated that growth of this grass was improved in non-sterile foreshore sand inoculated with *Glomus fasciculatus* and *G. macrocarpus* (Nicolson 1979). Hassouna and Wareing (1964) suggested that the vigour of *A. arenaria* in mobile dune areas may be due to the increased amount of nitrogen made available by non-symbiotic nitrogen-fixing bacteria, probably *Azotobacter*. Abdel Wahab (1975) could demonstrate that nitrogen-fixing *Bacillus* spp. were present in the rhizosphere of *A. arenaria*. Criticism on this hypothesis followed from the observation that the organic matter content of these areas was so low that organic carbon had to be added for N-fixation (Huiskes 1979), a conclusion that was later confirmed by Abdel Wahab and Wareing (1980), who found that nitrogen fixation was strongly limited by the supply of root exudates.

In their seminal paper, van der Putten et al. (1988) first discovered that the performance of *A. arenaria* was lower in non-sterile rhizosphere soil than in either sea floor soil or sterilised rhizosphere soil. Their experiment demonstrated that if any beneficial effect of microorganisms on the nutrition of *A. arenaria* was present, it was overshadowed by a strong growth-reducing biotic soil factor. Therefore, a new hypothesis was formulated, namely that *A. arenaria* needs a continual supply of fresh sand to stimulate the growth of new roots, not because of physical ageing or to obtain a sufficient amount of nutrients, but to escape from harmful soil biota. This has later been called the 'escape-hypothesis'. In this paper (van der Putten et al. 1988), roots and rhizosphere soil were investigated for the presence of plant-parasitic nematodes, but since only few were found, it was then supposed that these were not the main cause. Later experiments (van der Putten et al.

1993) demonstrated that later successional species were tolerant to the soil pathogens that accumulated in the rhizosphere of *A. arenaria*, thereby suggesting that these pathogens contributed to vegetation succession. In a first attempt to identify the growth-reducing soil factor, De Rooij - Van der Goes (1995) demonstrated that a combination of naturally occurring pathogenic fungi as well as large numbers of *Tylenchorhynchus ventralis* nematodes were capable of reducing *A. arenaria* growth, although the number of nematodes needed to obtain this effect was 80 times greater than in non-sterile dune soil. However, nematicide application enhanced growth of *A. arenaria*, strongly suggesting the involvement of plant-parasitic nematodes in the *Ammophila* problem (van der Putten et al. 1990, van der Putten and van der Stoel 1998). The observation that the start of a negative plant-soil feedback coincided with the colonisation of newly deposited sand by the endoparasitic nematodes *Heterodera arenaria* and *Pratylenchus* spp. further supported this idea (van der Stoel et al. 2002). A negative effect of the endoparasites *H. arenaria*, *P. penetrans* and *Meloidogyne maritima* was suggested (Brinkman et al. 2004), but a later study could not confirm that the combination of these three species reduced the performance of *A. arenaria* (Brinkman et al. 2005c). Further investigations suggested that interspecific competition between different endoparasitic nematodes might determine the eventual effect of each species on plant biomass (Brinkman et al. 2005a, b), with positive effects arising from the outcompeting of harmful species by less detrimental species, such as *H. arenaria* (van der Stoel and van der Putten 2006). The effect of each of the endoparasitic species furthermore depended strongly on their respective densities, leading to contrasting results of field and laboratory experiments (Brinkman et al. 2005a, b). It had earlier been demonstrated that mycorrhizal fungi could mitigate the negative effects of plant-parasitic nematodes on *A. breviligulata* (Little and Maun 1996), which turned out to be the case for *A. arenaria* as well (de la Peña et al. 2006), where AMF suppressed nematode infection and reproduction. Moreover, complex interactions between the endophytic fungus *Acremonium strictum* and the species *P. penetrans* and *P. dunensis* were discovered (Hol et al. 2007). Piskiewicz et al. (2008) investigated the extent to which the effect of eight different root-feeding nematode species, specific to coastal dunes, on growth of *A. arenaria* was controlled by either a full nematode community, microorganisms or microarthropods. A significant suppression of six out of eight nematode species by other soil organisms was

observed. Each of the six species performed worse in the presence of soil microorganisms, while two nematode species were controlled by the full nematode community, and only one nematode species was significantly controlled by microarthropods. Although capable of controlling root-feeding nematodes, greater reductions in shoot or root biomass were often observed in the presence of these other soil organisms, compared to when the nematode species were inoculated alone. However, in the case of *Pratylenchus* sp. the shoot biomass was significantly enhanced when other nematodes or arthropods where inoculated as well. It was shown that the suppression of *T. ventralis* by microorganisms was due to local interactions, potentially parasitism of the nematodes by bacteria and fungi (Piskiewicz et al. 2007, Piskiewicz et al. 2009).

Combined, these studies sketch one of the most detailed pictures of the variety and specificity of the different biotic interactions in the rhizosphere of a wild plant. Notwithstanding the complexity of these interactions, it is noteworthy to remember that their net effect in wild soils appears to be negative, indicating that mutualists and controlling organisms are generally not able to overcome the effects of antagonists (Brinkman et al. 2005c, Piskiewicz et al. 2009, van der Putten et al. 1988, van der Putten et al. 1993). Furthermore, in recent years, new evidence in favour of the original hypothesis of root efficiency of nutrient extraction has been put forward (Boudreau and Houle 2001, Day et al. 2004). The expansion of *A. arenaria* in lime- and iron-poor dunes has also been ascribed to the relatively higher availability of P when it is not sequestered into iron and aluminium phosphates, rendering the system N-limited. This gives *A. arenaria* an advantage because of the relatively efficient recycling of N from its own litter under these circumstances (Kooijman and Besse 2002). It thus seems that both biotic and abiotic factors have been considered as explanation for the *Ammophila* problem. However, researchers investigating the effects of soil biota have never explicitly considered variation in abiotic conditions in their experiments and vice versa. An experiment in which both the biotic and abiotic component of soil are varied independently is the only way to disentangle their relative contributions to the *Ammophila* problem.

As the cycle of trapping wind-blown sand, growing upwards and forming new roots in the newly deposited layer repeats itself on a yearly basis, *A. arenaria* plays an important role in the process of dune formation. Because

of this ability to fixate sand, *A. arenaria* has been used since historical times for erosion control in Europe. As early as 600 B.C., the Gauls undertook successful programs of seeding the dunes of Arcachon in order to prevent their capital cities of being inundated with sand (Green 1965). The earliest reference to sand fixation in northern Europe was in Germany in 1316 (Whitfield and Brown 1948). The main dune cultivation, however, developed in the 17th and 18th centuries (Green 1965). In France *A. arenaria* was planted first to build a foredune and then to stabilise the main dune complex. Transplantation of the culms proved to be more effective than seeding the dunes. After primary fixation with the grass the secondary plantings were begun, using *Pinus* seeds and *Cytisus scoparius* as nurse plant (Green 1965). Dunes have been fixated with *A. arenaria* in Germany, Denmark, Belgium, the Netherlands, England, Scotland and Ireland (Green 1965). Methods of planting are still being optimised as the practice of dune stabilisation with *A. arenaria* is still performed widely throughout Europe (van der Putten 1990, van der Putten and Kloosterman 1991).

Because sand fixation with *A. arenaria* had proven so successful in Europe, man has introduced this species in countries across the world. *A. arenaria* currently occurs in Australia, New Zealand, South Africa, Palestine, India, the Falkland Islands, Chile and the west coast of the USA (Green 1965, Hilton et al. 2005, van der Putten et al. 2005). In Australia (Heyligers 1985), New Zealand (Hilton et al. 2005) and the USA (Wiedemann and Pickart 1996), the species is now considered invasive. In the USA, *A. arenaria* is known to have led to the formation of a high and wide foredune in places where no foredune was originally present (Wiedemann and Pickart 1996). *A. arenaria* furthermore has displaced entire native plant communities, and has drastically reduced their habitat (Pickart 1997, Wiedemann and Pickart 1996), thereby impacting six federally endangered plant species as well as the threatened western snowy plover *Charadrius alexandrinus nivosus*, a shorebird that nests in open areas on the strand (Pickart 1997). Similar effects are observed in New Zealand, where dunes dominated by *A. arenaria* are higher and steeper than dunes formed in association with indigenous foredune species, such as *Desmoschoenus spiralis* and *Spinifex sericeus*. The complete displacement of both *Desmoschoenus* and *Austrofestuca* by *A. arenaria* has been attributed to increased rates of sand accretion, that lead to levels of burial beyond the tolerance of native dune species (Hilton et al.

2005). In contrast to the exponential spread of *A. arenaria* in other introduced ranges, in South Africa the species seems restricted to the original sites of plantation, probably because of limitation by water availability (Peter 2000). Although it is currently not yet considered invasive, the persistence of the planted populations and the known potential for invasion have raised concern and the question for more research (Hertling and Lubke 2000).

Although the devastating effects of *A. arenaria* invasion on some of the native communities are obvious, the actual mechanism of invasiveness is less clear. Given the above-cited large amount of study on the role of soil biota in the decline of *A. arenaria* in its native range, a logical hypothesis would be that the antagonistic soil organisms are lacking or less abundant in the introduced ranges. This is the well-known Enemy Release Hypothesis (ERH), which attributes the success of invasive plant species to the release of their coevolved natural enemies (Maron and Vila 2001, Keane and Crawley 2002). The majority of studies have, however, tested the ERH in the context of aboveground enemies. Recent meta-analyses (Chun et al. 2010, Liu and Stiling 2006) have shown that plants indeed have lower numbers of herbivorous insects in the introduced than in the native ranges, although this decrease in damage does not always lead to enhanced plant performance, concluding that enemy release is a probable mechanism of invasiveness in some cases. Two studies investigating the impact of herbivores on native and introduced plants through herbivore exclusion experiments, however, both showed support for the ERH (Schierenbeck et al. 1994, DeWalt et al. 2004). The ERH has been tested for *A. arenaria* and its soil-borne enemies in three studies (Beckstead and Parker 2003, Knevel et al. 2004, van der Putten et al. 2005). None of these, however, yielded unequivocal evidence that *A. arenaria* experiences less root-associated antagonists in its introduced ranges.

The relationship between *A. arenaria* and its root-feeding nematodes has not only been compared between native and introduced ranges, but also between different populations from within the native range. In a cross-inoculation experiment, Schreck Reis et al. (2008) investigated the multiplication of *Helicotylenchus* n. sp. from Portugal and *Helicotylenchus pseudorobustus* from the Netherlands on *A. arenaria* plants from either geographic origin. The authors hypothesised that reproduction of local root-feeding nematodes would be better on the local than on the non-local plant

population, because of local adaptation. In contrast to this hypothesis, the Portuguese *Helicotylenchus* species performed better on the Dutch plant population. De la Peña et al. (2009) performed a similar experiment. *A. arenaria* plants from each of four different populations were inoculated with each of four populations of *Pratylenchus* nematodes. Populations of *P. dunensis* were obtained from Oostvoorne (the Netherlands) and Comporta (Portugal). Populations of *P. brzeskii* originated from Biarritz (France) and Ynyslas (UK). Plant populations originated from Oostvoorne, Blakeney Point (UK), Ynyslas and Comporta. Idiosyncratic responses of nematodes to plant populations were observed, but none of the combinations provided evidence of local adaptation. On the contrary, one of the nematode populations performed significantly worse on the sympatric host. The populations of *A. arenaria* used in both studies are known to differ genetically (Rodríguez-Echeverría et al. 2008a). Because both experiments were conducted under the constant environmental conditions of the laboratory, they prove that broad-sense genetic differences between *A. arenaria* populations mediated the performance of the root-feeding nematodes. Interestingly, both studies found better performance of nematodes on non-local plant populations. These results are in line with the observation that *A. arenaria* does not experience less root-feeding nematodes in its introduced ranges, and might point at a more general pattern.

In glaring contrast to this extensive knowledge on the ecology of the organisms living in the rhizosphere of *A. arenaria* stands the very limited information about the interactions of this plant with aboveground organisms. However, a considerable number of herbivorous invertebrate species have been reported to feed on *A. arenaria*, including some highly specialised ones (Huiskes 1979, Weeda et al. 1994). Tussocks of *A. arenaria* furthermore provide shelter to a range of predatory arthropods, among which some spider species that are highly adapted to life in the drifting sand. The slowly decomposing litter that accumulates in the base of *A. arenaria* tussocks forms the food source of woodlice and other decomposers. In short, a full aboveground food web is centred around this single foundation plant species, that has received no attention in the ecological literature so far. Interestingly, two studies on the invertebrates associated with *A. arenaria* were conducted in the introduced range, to assess how local animal communities are impacted by this invader. Slobodchikoff and Doyen (1977)

found that while dune stabilisation by native plants increased sand burrowing arthropod diversity and abundance, even low percentages of *A. arenaria* cover strongly depressed arthropod populations, leading to fewer species and a smaller number of rare species. Denuded coastal foredunes in Australia that had been stabilised with *A. arenaria* two decades ago had a higher abundance of Diptera and two ant morphospecies and a lower abundance of Isopoda than foredunes with no anthropogenic disturbance (Webb et al. 2000).

There are several reasons why the dune grass *A. arenaria* forms a suitable model system to address some of the issues raised in the previous sections. Firstly, working with *A. arenaria* has some valuable practical advantages, because of the nature of the community and habitat to which this species belongs. Throughout large parts of its habitat, *A. arenaria* is the single occurring plant species. Sand is furthermore a relatively simple soil type, compared to for example forest soil, with a less species-rich soil community. In their natural state, the dynamic sand dunes where *A. arenaria* thrives largely lack vertebrate grazers, and this grass is known to be avoided by them (Huiskes 1979). If the aim is to start unravelling the full complexity of the multiple interactions between above- and belowground organisms in their natural context, such a system consisting of a single plant species growing in a structurally simple soil matrix might provide a good starting point. Secondly, the large body of literature on the involvement of soil biota in the specific ecology of this grass provides an invaluable source of existing knowledge to build upon. Thirdly, it is known that populations of *A. arenaria* are genetically different, and that these differences have consequences for different root-feeding organisms. Together with its functionally diverse aboveground invertebrate community, this makes *A. arenaria* an interesting species to assess the above- and belowground community phenotypes of these genotypes. Finally, the effects of non-local genotypes on aboveground natural enemies might raise novel hypotheses about the invasiveness of this species in the introduced range.

However, as this thesis will repeatedly demonstrate, even the *A. arenaria* system still harbours a diversity of soil and aboveground organisms large enough to create a considerable complexity of interactions. The unravelling of this complexity has proven and will continue to prove challenging.

1.8 Objectives and outline of the thesis

The aim of this thesis was to expand the current knowledge on the plant-mediated interactions occurring between above- and belowground biota and to explore implications of abiotic and plant genetic variation for these interactions. *A. arenaria* was used as a model species to address these issues in the context of sand stabilisation and the *Ammophila* problem. The aim was to combine factorial experimentation in the laboratory under controlled conditions with field manipulation and a field survey. A schematic overview of the different topics addressed in this thesis is given in figure 1.3.

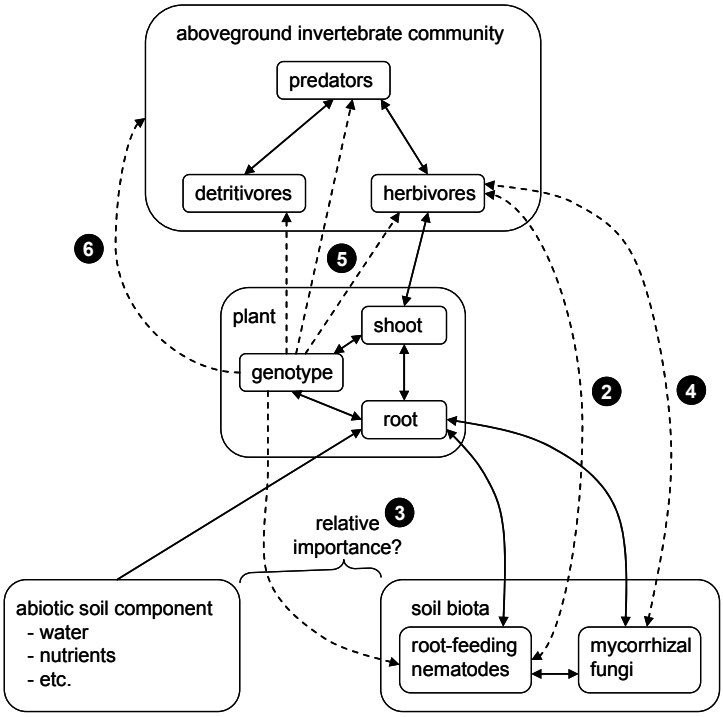


Figure 1.3 Schematic representation of interactions between above- and belowground components in the *A. arenaria* system. Solid arrows: direct interactions. Dashed arrows: indirect interactions, mediated by plant traits. Numbers correspond with the chapters in which particular relationships are investigated. Note that this scheme focuses on the interactions that are studied in this thesis, and is, hence, not exhaustive. For example, detritivores can affect plants through their effects on litter quality and nutrient availability.

In **chapter 2** I investigated whether a community of naturally occurring root-feeding nematode species could affect the performance of the specialist aphid *Schizaphis rufula*, and vice versa, in a constant laboratory environment. I subsequently determined whether the observed interactions in the laboratory led to predictable distributions of both root-feeding nematodes and *S. rufula* aphids under variable environmental circumstances in the field, or whether other factors were more important there. Both positive and negative interactions between above- and belowground herbivores have been demonstrated in either direction, depending on the particular study system (section 1.3). Therefore, it is difficult to hypothesise about the sign and strength of such interactions in our study system. However, I hypothesise that any interaction between aphids and nematodes would be more pronounced in the constant environment of the laboratory.

In **chapter 3**, non-sterile soil from dynamic and stabilised dunes was cross-inoculated into sterile soil of either type. A small soil volume was used as inoculum, so that the abiotic properties of the bulk soil were not affected. The organisms present in the inoculum were given time to proliferate in the treatment soils. This setup was replicated across three geographically separated dune areas. On these soils, *A. arenaria* seedlings were grown with or without *S. rufula* aphids. This setup allowed to decouple the effects of the abiotic and biotic soil component on plant growth and aphid performance, and to test which effects were detectable across different dune systems. I hypothesise that the input of additional nutrients by the deposited sand causes the performance of both plants and aphids to be higher for dynamic than for stabilised sterile dune soils. Because the accumulation of plant pathogens is expected to result in reduced plant growth, I hypothesise that aphids should perform worse on inoculated soils, especially those with biota from stabilised dunes. Given the usual spatial variability of soil nutrients and organisms, we expect the magnitude of these effects to differ between regions.

In **chapter 4**, seedlings of *A. arenaria* received *S. rufula* aphids and/or mycorrhizal fungi to investigate the effect of these mycorrhizal fungi on plant growth and aphid performance. Several population parameters of the aphids and plant characteristics were determined in order to identify possible mechanisms causing effects. I hypothesise that mycorrhizal fungi benefit plant growth, which in turn leads to an increased performance of the aphids.

In **chapter 5**, six populations of *A. arenaria* from locations throughout the native range were installed in a common garden in the field and exposed to natural colonisation by above- and belowground invertebrate species. The aim of this field experiment was to determine whether plant genotype affected the occurrence of individual above- and belowground invertebrate species, and whether correlations between these occurrences across genotypes could be detected that led to a pattern of covarying clusters of species. The average correlation between above- and belowground invertebrate species was of specific interest, as this might have consequences for the study of resistance trade-offs and the issue of pairwise versus diffuse coevolution. Based on previous literature, I hypothesise that covariation between aboveground invertebrate species will be positive on average. Because belowground organisms are currently lacking in studies of species' covariation across plant genotypes, it is difficult to predict whether root and shoot invertebrates will respond similarly, independently or oppositely to plant genotype. If plants possess a generalised defence mechanism, I hypothesise that responses will be similar. If root and shoot invertebrates select on different and independent plant traits, responses are expected to be independent. If some trade-off exists between the plant's susceptibility towards above- and belowground invertebrates, I hypothesise that responses will be opposite. Given the complexity and diversity of traits that determine a plant's associations with invertebrates, either option is plausible.

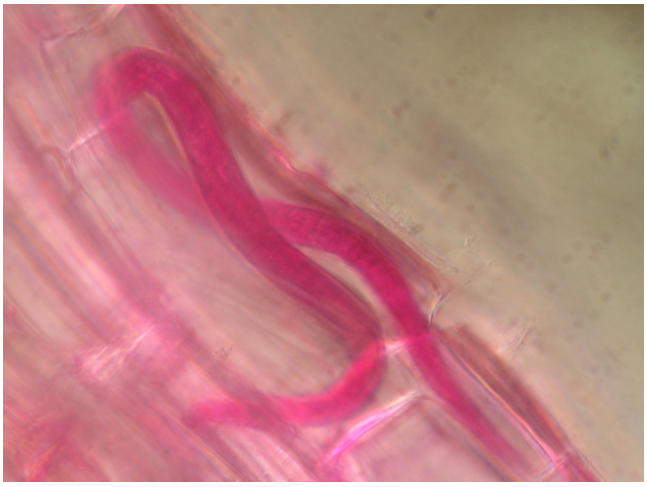
In **chapter 6** I have investigated whether individual responses of the aboveground invertebrate species to plant genotype, investigated in chapter 5, scale up to the level of local diversity. I specifically assessed how plant genotype affected the alpha diversity of herbivores, predators and omnivores, and detritivores as groups, and how this affected the total alpha diversity. I furthermore investigated whether relationships could be detected between the invertebrate species turnover between plant populations and their geographical or selectively neutral genetic distance. Finally, body size ratio based food webs were constructed and it was determined whether several statistics of these webs differed between genotypes. The aim was to find out whether the introduction of non-local genotypes of *A. arenaria* had effects on the local aboveground invertebrate community, which might yield new insights into the invasiveness of this species elsewhere in the world. I

hypothesise that the introduction of *A. arenaria* populations into ecosystems with native stands of *A. arenaria*, can affect the diversity and food web structure of the local invertebrate community. I further hypothesise that populations from distant locations affect the local diversity to a larger extent and that species turnover increases with distance between populations.

In **chapter 7**, the results presented in the previous chapters are discussed in a broader context. The implications of these results for conservation of the studied species, as well as for the research on aboveground-belowground interactions in general, are explored. The chapter ends with a number of candidate mechanisms for some of the observed patterns and proposes a few concrete suggestions for future experimentation.

2 Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field*

Martijn L. Vandegehuchte, Eduardo de la Peña and Dries Bonte



Example of a root-feeding nematode as seen under the microscope after the root has been stained with acid fuchsin.

---

* Adapted from: Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. - Oikos 119: 1011-1019.

2.1 Abstract

Over the past decades a growing body of literature has presented proof of the possible interactions between foliar and root herbivores. These effects can be positive, negative or neutral in either direction, depending on the species and the involved mechanism. Most of these studies however concern experiments under controlled conditions. Whether these interactions affect the distribution of herbivores under natural conditions still largely remains an open question. This study examined interactions between root-feeding nematodes and shoot-feeding aphids on *Ammophila arenaria* in the laboratory. We subsequently addressed the question whether expectations from this experiment are reflected in correlations between plant-related variables and the abundance of both herbivores in the field. We demonstrated that nematodes and aphids can negatively affect each other in a controlled microcosm. In the field, however, no significant correlations between nematode and aphid abundances could be detected. There, shorter plants with a more vital leaf set and a higher root density supported the highest numbers of aphids. Plants with a lower root density and higher root vitality held more migratory endoparasitic nematodes, while more nematode cysts were found among roots with a low vitality. Certain aspects of plant morphology can furthermore relate to above- and belowground herbivores in the opposite direction, such as root density in this case. This study suggests that effects of root herbivores on foliar herbivores or vice versa seem to be blurred in a field situation where other variables related to plant vitality and water content structure the herbivore populations. Therefore, caution should be used in generalising the prevalence of these interactions between the above- and belowground fauna, based solely on laboratory experiments.

2.2 Introduction

Over the past decades it has become clear amongst ecologists that plants in terrestrial ecosystems enable different types of interactions and feedbacks between the above- and belowground community. These interactions can furthermore affect biodiversity and ecosystem processes (reviewed by van der Putten et al. 2001). Root-feeding organisms, pathogens or root mutualists such as mycorrhizae can have direct effects on the performance of plants and as a consequence on that of foliar herbivores and potentially their predators (Wardle et al. 2004). Aboveground herbivores have furthermore been proven beneficial as well as adverse for their belowground counterparts (Kaplan et al. 2008, Masters et al. 1993, Soler et al. 2007a, Tindall and Stout 2001, van Dam et al. 2003). While the mechanisms behind the interactions between foliar and root herbivores were explained in terms of water stress, primary chemistry and available biomass in early studies (Masters et al. 1993), recent studies highlighted the importance of plant secondary metabolism as an explanation of both positive and negative feedbacks (reviewed by van Dam et al. 2003).

Most of the above-mentioned studies are based on laboratory experiments with potted plants. A wide variety of at first sight unrelated environmental actors can cause the same metabolic response in plants (White 1984). Therefore, it seems hard to predict whether the effect – if any – of herbivores on other herbivores at the opposite side of the soil surface will be relevant or detectable compared to that of other environmental agents. Only a few studies have investigated whether predictions from controlled experiments on above- and belowground herbivore interactions hold in field situations. These either involved artificial field manipulations (Kaplan et al. 2009, Moran and Whitham 1990) or were conducted in agricultural systems (Kaplan et al. 2009, but see Moran and Whitham 1990). Because agricultural systems have artificially high levels of herbivory due to additional nitrogen fertilisation and unnatural densities of host plants (White 1978), it is unlikely that these findings reflect patterns from more natural systems.

In this study Marram grass, *Ammophila arenaria*, is used as a model species both in a laboratory experiment and a large field survey. This is a

dominant grass species of dynamic sand dunes and it is distributed along all European coasts south of latitude 63°N (Huiskes 1979). The plant grows most vigorously in the open habitat of mobile dunes and tolerates extreme wind and sun exposure and wide fluctuations of soil temperature (Huiskes 1979). *A. arenaria* displays strong growth as long as the plant is regularly buried with windblown sand. As soon as regular sand burial ceases, the plant starts to degenerate and decreases in shoot weight per plant and abundance per unit area (Eldred and Maun 1982).

The interactions occurring in the rhizosphere of dune grasses, and of *A. arenaria* in particular, form one of the best documented illustrations of the influence of root feeders on plant community development (van der Putten et al. 2001, Zoon et al. 1993). The soil and rhizosphere of plants in coastal dunes contain an ample variety of organisms, including root-feeding nematodes, that contribute to the plant–soil feedbacks that modify the fitness of competing plant species over the course of succession (van der Stoel et al. 2002). Therefore, the successional dynamics of the plant community, in particular the decline of species in favour of later successional species, are linked to the net effect of different soil-borne organisms. Since investigating the whole soil community was not practically feasible, we focused on the occurrence of root-feeding nematodes that are known from literature to be specific and abundant on *A. arenaria* (de la Peña et al. 2007, van der Stoel and van der Putten 2006). Previous studies in the sampling area (de la Peña et al. 2007) revealed that most abundant root-feeding nematodes inside *A. arenaria* roots belonged to the genus *Pratylenchus*, which is a group of migratory endoparasitic nematodes that penetrate into the roots of the plant and move through and feed on the cortex cells. The cyst forming nematode *Heterodera arenaria* is also present in abundant numbers in this area. This is a sedentary endoparasitic species which penetrates the root, establishes a feeding site and produces persistent forms (cysts) that protect the eggs. After one growing season these cysts usually get detached from the roots and accumulate in the soil. In contrast to *Pratylenchus* species (de la Peña et al. 2008, Seliskar and Huettel 1993), *H. arenaria* is shown to have little negative effect on the growth of its host plant (van der Stoel and van der Putten 2006). In addition to *Pratylenchus* spp., juvenile stages of *H. arenaria* and of root knot nematodes of the genus *Meloidogyne* can be found within *A. arenaria* roots.

Schizaphis rufula is an aphid species known to live specifically on leaves of *A. arenaria* and *Elymus arenarius* in sand dune areas. The leaves of the plant can turn yellowish brown as a result of the infestation (Heie 1992).

We conducted a fully crossed laboratory experiment to assess the potential for interactions between nematodes and aphids under controlled circumstances. We subsequently addressed the question whether expectations based on this manipulation experiment would be reflected in the abundances of both herbivore groups in the field. If so, we hypothesised that herbivore–plant feedbacks would result in correlated abundances of both below- and aboveground herbivores. The foliar herbivore could attain higher numbers on growing plants with a more vital leaf and root set (plant vigour hypothesis, Dhileepan 2004, Price 1991) or on plants with less vital senescing leaves (plant stress hypothesis, White 1978), depending on whether it is a 'flush feeder' or 'senescence feeder' respectively (White 1978, White 2009). Both types of plant tissue are characterised by an increased mobilisation of nitrogen, either towards the growing shoot, or out of the senescing leaf, that is beneficial to herbivores. Root-feeding nematodes are expected to colonise the plant in early spring (van der Stoel et al. 2002), before the first appearance of aphids in June (unpubl.). If nematodes are causing stress to the plant, then we expect a negative correlation between below- and aboveground herbivores under the plant vigour hypothesis and a positive one under the plant stress hypothesis. In case other stressors have a stronger impact on plant vitality, independent and uncorrelated responses to plant quality are expected. Aphids might positively affect nematodes by increasing the transport of nutrients or minerals to the root system (Johnson et al. 2009, Kaplan et al. 2008), or have a negative impact by decreasing plant productivity (Masters et al. 1993, Moran and Whitham 1990). Negative effects in both directions could be expected if one herbivore elicits some plant defence response that spreads to the other components of the plant (Bezemer and Van Dam 2005). Analogously, the destruction of production sites of such defences in one plant part can lead to lowered defence of other plant parts, leading to positive interactions (Kaplan et al. 2008).

2.3 Material and methods

2.3.1 Controlled experiment

Seeds of *A. arenaria* were obtained from the Westhoek nature reserve (Belgium). They were surface sterilised by submersing in 4% household bleach solution for 5 minutes, rinsing 10 times with demineralised water, submersing in 10% ethanol for 5 minutes and rinsing another 10 times with demineralised water (adapted from Sauer and Burroughs 1986). This sterilisation method effectively eliminates horizontally transmitted fungi that could otherwise colonise the young seedling. No endophytic fungi were detected in the endosperm of seeds from the sampled plant population (M. Omacini and E. de la Peña, unpublished result). Seeds were subsequently germinated at a light regime of 9/15 hours dark/light in plastic 1-l pots filled with 190 cm^3 of sterile dune sand that was autoclaved for 1 h at 120°C and 1 atm. The sand was saturated with demineralised water. Plastic foil that covered the pots was perforated to allow of enough ventilation. Moisture level was reset to near saturation daily.

Nematodes were extracted using a modification of the Baermann technique (Whitehead and Hemming 1965). Roots of vigorous *A. arenaria* plants were collected in the Westhoek nature reserve and placed on trays. The water was removed from the trays daily and poured into glass columns, after which trays were refilled with fresh water. Columns were left overnight so that the nematodes could sink, and then decanted leaving 200 ml of liquid in the columns. Nematodes were harvested daily according to this method. The concentration of nematodes in the glass columns thus gradually increased. After one week, the columns were emptied into a single recipient, which was kept in a fridge at 8 °C. Nematodes were counted in five 10 ml subsamples of this final solution, and averaged over those subsamples to calculate the number of nematodes per ml. The nematode community at the field site where the roots were harvested consisted of 53.75% *Pratylenchus brzeskii*, 15.62% *Tylenchorhynchus ventralis*, 15.62% *Meloidogyne* sp., 12.5% *Heterodera* sp., 4.25% *Paratylenchus* sp., 3.125% *Rotylenchus* sp., 3.125% *Filenchus* sp. and 2% other species.

Nineteen days after the seeds were placed on the sand to germinate, two freshly germinated seedlings were selected and transplanted to each of 40 plastic pots (1 l) filled with 550 g of autoclaved dune sand. Immediately after transplantation, half of the pots were inoculated with 15 ml of nematode inoculum, containing 552 nematodes on average. This corresponds with the higher densities observed in the field (E. de la Peña, unpublished data). This relatively high yet realistic density was used in order to assure that a considerable proportion of the nematodes infected the roots. The other half of the pots received 15 ml of tap water as a control. Pots were sealed with a piece of fine mesh cloth and placed under a 9/15 hours dark/light regime. Seventy-five ml of half-strength Hoagland's nutrient solution was added four days after transplantation. From then onwards all pots alternately received 50 ml of tap water and 50 ml of half-strength Hoagland's nutrient solution at three-day intervals. Pots were randomised on the growth bench twice a week. Twenty-four days after nematode inoculation, all pots were trimmed down to one seeding. Half of the nematode inoculated plants and half of the other plants received a single newborn first instar of *S. rufula.* The four treatment combinations thus were control (C), nematodes (N), aphids (A) and both aphids and nematodes (N+A), with ten replicate plants per combination. Aphids were obtained from a parthenogenetic lab culture that was propagated from one wild individual from the Westhoek nature reserve. Aphids were counted daily to determine a population growth curve for each replicate. Previous experiments with this system have shown that populations usually grow to a peak density after which aphid numbers rapidly decline. As soon as the last aphid of a population died, the plant was uprooted and root and shoot fresh weight were obtained. Roots were stored at 4°C until the end of the experiment. At day 40 the experiment was ended and all remaining aphid populations were collected and plants were uprooted. Root and shoot fresh weight were determined, after which all roots were stained with acid fuchsin, a dye that stains animal and fungal tissue (Baker and Gowen 1996). Because of the relatively small size of the roots, stained nematodes could be counted in situ in the complete root system, using large microscope slides (76 × 40 mm). Of the 20 first instar aphids that were added to the plants, four died before becoming adult. These data points were omitted from all analyses. Of the 16 plants with aphids, six contained nematodes in their root system, and 10 were nematode-free.

The effects of nematode and aphid addition on both root and shoot fresh weight were analysed by means of a general linear model, after which the assumptions about residuals were confirmed. The effect of aphid addition on the number of nematodes in the roots was assessed through a generalised linear mixed model with aphid treatment as categorical predictor and root fresh weight as a covariate. The effect of nematode inoculation on the population dynamics of aphids through time was tested by means of a repeated measures analysis with individual plant as subject (generalised estimating equation, GEE). We included nematode treatment, time (days), higher order power terms of time (up to sixth order) and all interactions as predictors. This allowed modelling the different maxima and minima in aphid populations that were observed. Shoot fresh weight was included as a covariate. A stepwise backward deletion of non-significant terms at the $p = 0.05$ level was conducted. All generalised models used a Poisson distribution and contained a correction for the observed overdispersion.

2.3.2 Field survey

From August until September 2007, a sampling campaign was held in six different sites in the Westhoek nature reserve, three of which are situated in the foredunes close to the coast line and three within a large inland dynamic dune area. Each site was visited once during this time period, and sites were visited in a random order. Sampling was always done on sunny days with no rain, to minimise climatic variation. At each site, 20 stands of *A. arenaria* were chosen, covering the spectrum from vital to partly degenerated and from large to small tussocks. Each selected stand was then swept five times with an insect net to catch aphids. The diameter and perimeter of the tussock and the length of the highest leaf were measured and a circular part of the tussock with a diameter of 20 cm was cut out at ground level. If the plant was small and had a diameter smaller than 20 cm, the entire plant was cut off. Since the number of aphids remaining in these entire tussocks after sweep netting was negligible compared to the numbers in the net, sweep netting seems to be an adequate method to quantify aphids in this particular system. The aboveground diameter of the tussock (cm) was used as a measure of plant size. Plant height was equated with the length of the highest leaf. Within a radius of 2.5 m around each sampled stand, the number of *A. arenaria* stands and the perimeter of each stand were

determined. The surface of each stand (cm^2) within this radius was derived from its perimeter that was assumed to approximate a circle. The surfaces of all stands were summed and this sum was divided by the surface of the circle, to calculate the proportion of the area within the radius occupied by *A. arenaria*. This proportion serves as an estimate of the relative amount of suitable habitat cover in the local environment of the sampled plant. For each sampled stand, a soil sample of 1 l including roots was taken at the point where the leaves were cut. Leaf and soil samples were transferred to the lab. Dead leaves were separated from living leaves of *A. arenaria* and living leaves of other plant species and the different types of leaves were weighed. Dead leaves were pooled because they were often decomposed too far to identify the species. An aboveground vitality measure was calculated as the weight of living leaves of *A. arenaria* divided by the summed weight of living leaves of *A. arenaria* and dead leaves. The soil samples were sieved over a 4 mm mesh sieve with 4 l of tap water. Roots were collected from the sieve and if necessary roots from *A. arenaria* were separated from roots of other plant species. The root density was determined as the fresh weight of the roots in grams per litre of soil. The fresh roots were then cut into pieces of approximately 1 cm length and stained according to the above described method. For each sample, 30 randomly chosen root fragments were inspected under a microscope. The length of each fragment was measured in millimetres, the fragment was categorised as vital or degenerate, with a fragment that had lost more than half of its cortex being denoted as degenerate, and the number of nematodes in the fragment was recorded. The sum of the nematode counts across the 30 fragments was divided by the sum of the lengths of the fragments in cm, to obtain the average number of nematodes per cm of root. A vitality measure for the root system was calculated as the proportion of vital fragments in each sample. The suspension of 1 l of soil in 4 l of tap water was decanted over a 180 µm mesh sieve. The sieved fraction was dried at room temperature and inspected with a stereomicroscope to count the number of cysts of *Heterodera* sp. within the entire sample. Insect nets were emptied in the lab and aphids were transferred to ethanol. Aphids were cleared and mounted according to the method of Hille Ris Lambers (1950) and were identified with the identification key by Heie (1980, 1982, 1986, 1992, 1994, 1995). The 120 samples contained a total of 1803 aphids.

Of these, 1797 belonged to the species *S. rufula* and the other six individuals were excluded from further analyses.

So for each of the 120 sampled *A. arenaria* stands (20 plants in each of six sites) six plant-related variables were recorded: leaf vitality, root vitality, root density, tussock diameter, local habitat cover and plant height. In addition three herbivore-related variables were considered: the number of aphids, the number of nematodes per cm of root and the number of cysts per litre of soil. This amounted to nine variables with 120 values each and the variable site with six levels.

To analyse the relationships between the different herbivores and their habitat, a double approach was followed. The general habitat-centred approach describes the variation in the available habitats and evaluates the position of the focal herbivores within that variation. The focal herbivore-centred approach attempts to predict abundances of the different herbivores from those habitat variables. These variables might be environmental factors as well as other herbivores.

The general habitat-centred approach consisted of an ordination by means of Nonmetric Multidimensional Scaling (NMS) of all variables, based on Sørensen distances. A random starting configuration was used and 50 runs were performed with real data for each number of dimensions, ranging from 1 to 6. A Monte Carlo test was performed with an additional 50 runs with randomised data for each dimensionality. Additional dimensions are considered useful if they reduce the final stress by 5 or more. The highest dimensionality that meets this criterion was chosen for the final solution. The stability criterion was set to 0.00001, with a maximum number of iterations of 500. Prior to the analysis, variables were relativised by variable totals. For an argumentation on the benefits of NMS compared to other ordination techniques, see McCune and Grace (2002).

The focal herbivore-centred approach consisted of multiple regression analyses. For each of the three herbivore-related variables, all other variables were combined to a set of eight predictors, including abundances of other herbivores. These explanatory variables were included as fixed effects in a generalised linear mixed model with site as random effect, and the abundance of the herbivore in question as response variable. Poisson models

with a correction for the observed overdispersion and Satterthwaite's approximation of the effective degrees of freedom were used in all cases. No interactions between variables were included. A backward stepwise selection procedure was followed in which predictors with a p-value larger than 0.05 were successively deleted, to obtain a final model for each response variable. For nematodes, total numbers in a sample were used as response variable, with the combined length of the 30 investigated root fragments as an offset variable.

2.4 Results

2.4.1 Controlled experiment

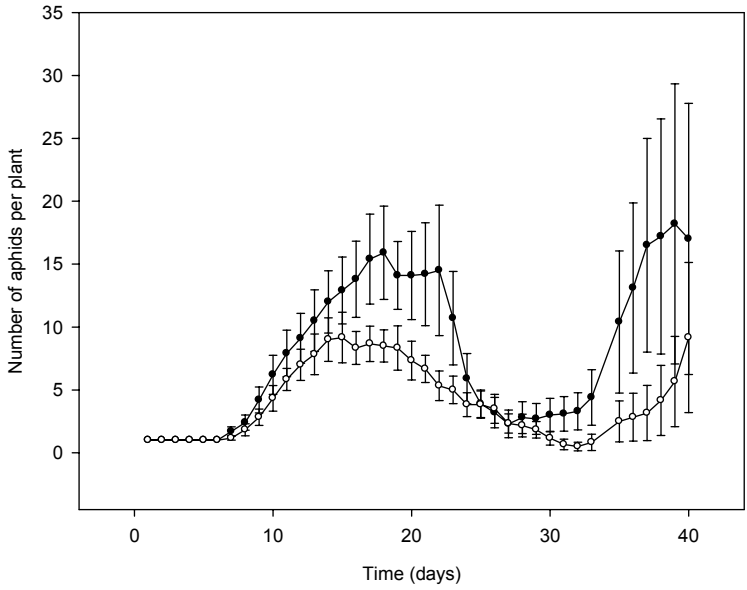


Figure 2.1 The growth of aphid populations over the course of time on plants with (open dots) or without (black dots) endoparasitic nematodes in the root system (average + SE).

There was a highly significant negative effect of aphid addition on root biomass ($F_{1,34}$ = 72.71, p < 0.0001). Plants that received no aphids produced on average 0.042 g of roots, while plants with aphids had an average root weight of 0.014 g (a decrease of 65.2%). Aphids also significantly depressed shoot growth ($F_{1,34}$ = 89.95, p < 0.0001), with shoots reaching an average weight of 0.049 g compared to 0.175 g for plants without aphids (a decrease of 72.1%). Nematodes did not significantly influence root or shoot biomass.

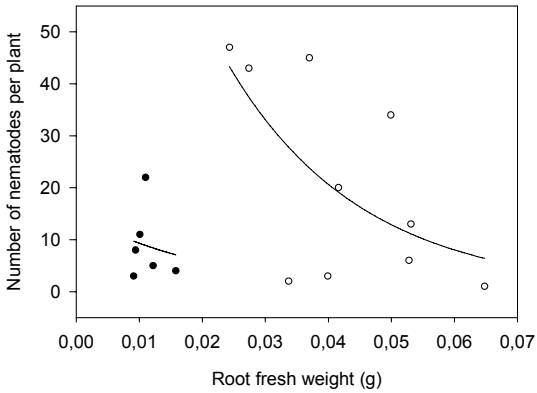


Figure 2.2 Relationship between root fresh weight (g) and number of endoparasitic nematodes in the root system for plants with (black dots) or without (open dots) aphids.

The development of aphid numbers through time differed significantly between plants with and without nematodes (Fig. 2.1). After stepwise deletion of non-significant terms, the final model retained time and all higher order power terms of time up to the fifth order (χ^2 = 6.49 or higher, p < 0.01), as well as nematode treatment (χ^2 = 4.9, p = 0.0268). There were no significant interactions between nematode treatment and any of the time power terms. This implies that the position in time of different minima and maxima in aphid numbers was not statistically different between treatments. In other words, population dynamics of aphids on plants with or without nematodes were similar, but lower numbers of aphids were attained in the presence of nematodes. Numbers of aphids on nematode inoculated plants can be predicted by the equation $A = \exp(-0.00000267 \times T^5 + 0.000299 \times T^4 - 0.01169 \times T^3 + 0.1839 \times T^2 - 0.9032 - T + 0.6431)$, with A = number of aphids

and T = number of days. For nematode-free plants, the equation is identical except for the addition of the constant 0.6615. The numbers of nematodes within the roots were significantly related to root weight ($F_{1,13}$ = 5.48, p = 0.0358) and the presence or absence of aphids ($F_{1,13}$ = 9.95, p = 0.0076). The interaction was not significant (Fig. 2.2). Within the different treatment groups, numbers of nematodes were higher on roots with lower biomass. Apart from this effect, nematodes reached higher numbers on plants without aphids.

2.4.2 Field survey: NMS

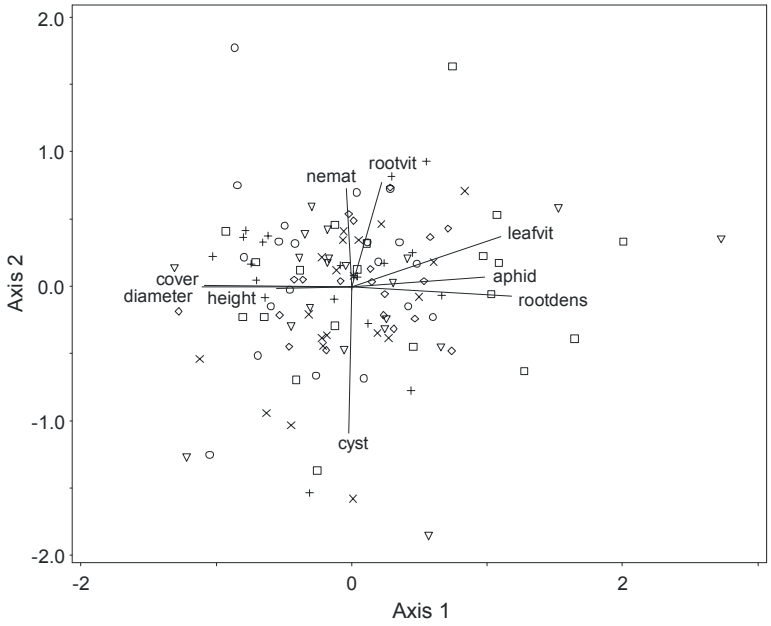


Figure 2.3 Nonmetric Multidimensional Scaling (NMS) joint plot. Each data point represents a single sampled *A. arenaria* stand. Different sites are indicated by different symbols. Individual variables are represented by lines that radiate from the centroid of the point cloud. The projection of a line on an axis represents the r^2 value between that variable and the axis. Legend: nemat = number of endoparasitic nematodes per cm of investigated root, cyst = number of cysts per litre of soil, aphid = number of aphids, leafvit = vitality of the leaf set, rootdens = density of the root system (g l^{-1}), height = plant height (cm), diameter = tussock diameter (cm), cover = proportion of *A. arenaria* cover within a 2.5 m radius, rootvit = vitality of the root system.

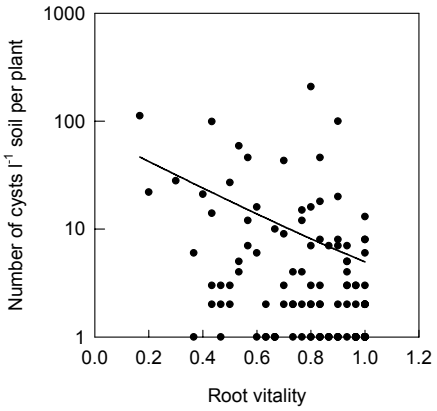


Figure 2.4 Correlation between root vitality of *A. arenaria* stand (proportion of vital root fragments within a random sample of 30 fragments) and number of cysts within a soil sample of 1 l taken beneath that stand. Note that the response variable was y + 1 transformed to visualise zeroes on a log scale. The line represents the final model.

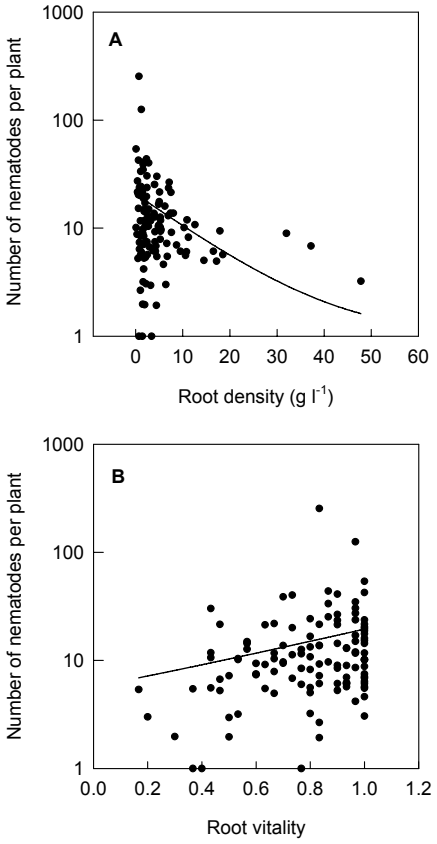


Figure 2.5 Correlation between (A) root density and (B) root vitality (proportion of vital root fragments within a random sample of 30 fragments) of *A. arenaria* stand and number of endoparasitic nematodes summed across 30 randomly chosen root fragments of 1 cm length from that stand. Note that the response variable was y + 1 transformed to visualise zeroes on a log scale. The lines represent the final model with the other predictor variables substituted by their average value over all samples.

A three-dimensional solution was retained, which means that three gradients captured most of the variation in sampled *A. arenaria* stands. The

three axes contained 49.5%, 32.6% and 10.2% of the total variance (92.3% cumulative). Further axes resulted only in a comparably small reduction of final stress. The final solution lowered the stress to 10.98646, and reached the desired instability of 0.00001 after 141 iterations. The Monte Carlo test yielded a p-value of 0.0196, i.e. the probability of obtaining an equal or lower stress than the observed stress by chance. Ecological datasets tend to produce solutions with stress between 10 and 20. Values in the lower half of this range are considered satisfactory (McCune and Grace 2002).

In the ordination diagram (Fig. 2.3), all sampled plants are plotted against the two axes that explain most of the variation. Variables are represented by lines that radiate from the centroid of the ordination scores. The r^2 value between a variable and an axis can be visualised by projecting the line perpendicularly onto the axis of interest. Plants from the six different sampling sites are spread evenly over the diagram, which means that the variation within sites is generally larger than between sites. All of the included variables correlate considerably with at least one of the axes. This implies that all of them contribute to the observed variation in sampled plants. The latter moreover form gradients along the axes rather than clusters, which confirms the sampling campaign succeeded in covering the complete spectrum of variation in plants. Aphid numbers correlate positively with leaf vitality and root density, and negatively with plant height and the strongly correlated variables vegetation cover and tussock diameter. These variables correlate with the first axis. Cyst number correlates negatively with root vitality and nematode abundance, along the second axis.

2.4.3 Field survey: multiple regression analyses

Only root vitality was retained as a significant predictor ($F_{1,118} = 7.36$, $p = 0.0077$) of cyst abundance, with more cysts found on less vital roots (Fig. 2.4). A significant positive correlation of root vitality ($F_{1,116.7} = 4.20$, $p = 0.0428$) and negative correlation of root density ($F_{1,116.8} = 4.84$, $p = 0.0298$) with numbers of plant-parasitic nematodes were observed (Fig. 2.5). Root density ($F_{1,115.5} = 16.39$, $p < 0.0001$) and leaf vitality ($F_{1,113.9} = 20.02$, $p < 0.0001$) were retained as significant predictors of high aphid abundance, while aphid numbers were negatively related to plant height ($F_{1,111.9} = 23.28$, $p < 0.0001$, Fig. 2.6).

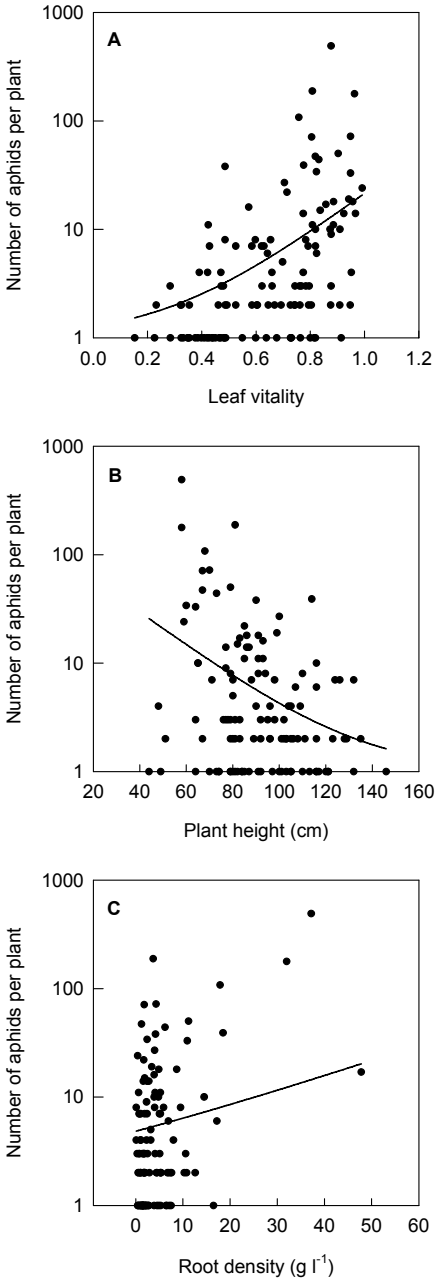


Figure 2.6 Correlation between (A) leaf vitality (weight of living leaves to total shoot weight ratio of subsample), (B) height and (C) root density of *A. arenaria* stand and number of aphids caught on that stand by sweep-netting. Note that the response variable was y + 1 transformed to visualise zeroes on a log scale. The lines represent the final model with the other predictor variables substituted by their average value over all samples.

Note that in general, the results of the multiple regressions correspond well with the NMS ordination diagram. This means that both the general habitat-centred approach and the focal herbivore-centred approach lead to same conclusions. The main difference is that the multiple regressions allow determining which of the different correlated variables are actually able to predict the numbers of each herbivore. Although aphids correlate most with leaf vitality, root density, plant height, plant diameter and vegetation cover, the latter two are less suited to predict their numbers. Cysts, nematodes and root vitality all correlate with the second axis but only root vitality is a significant predictor of both their abundances.

2.5 Discussion

The laboratory experiment clearly demonstrated that root-feeding nematodes and aphids of Marram grass can negatively affect one another. Aphids reached higher population densities in the absence of nematodes. Nematode numbers in roots were highest in small root systems of plants without aphids. In the field survey however, of all the tested variables, only the strictly plant-related variables were significantly related to both above- and belowground herbivore abundances. A negative correlation between root vitality and cyst numbers was found, while numbers of endoparasitic nematodes correlated positively with root vitality and negatively with root density of *A. arenaria*. The abundance of *S. rufula* aphids on the other hand showed a positive correlation with root density and leaf vitality and a negative correlation with plant height. The abundance of none of the studied herbivore groups was significantly related to that of any of the other herbivore groups, neither to the spatial context of the host plant. Above- and belowground herbivore abundances in the field are therefore determined by different characteristics of their mutual host plant rather than by each other.

Interactions between above- and belowground herbivores are highly diverse and hence hard to predict. The literature provides evidence of mutualism (Johnson et al. 2009, Kaplan et al. 2008) as well as competition (Staley et al. 2007, Tindall and Stout 2001). Furthermore ammensalism, the combination of a negative and neutral effect (Blossey and Hunt-Joshi 2003, Moran and Whitham 1990) and contramensalism, the combination of a

positive and a negative effect (Blossey and Hunt-Joshi 2003, De Deyn et al. 2007, Masters et al. 1993, van Dam et al. 2003), have been reported for root and shoot herbivores in either direction. The underlying mechanisms are equally diverse. Positive effects of root herbivores on shoot herbivores were explained through the induction of water stress, causing an increase in the amount of soluble nitrogen in the leaves (Masters 1995), or by root herbivores damaging production sites of secondary metabolites in the roots (Kaplan et al. 2008). Positive effects on root herbivores were attributed to shoot herbivores causing an increase in sink strength of the roots (Kaplan et al. 2008) or an increase in root minerals (Johnson et al. 2009). Most negative effects, in either direction, are imputed to the induction of chemical defences (Bezemer and Van Dam 2005). Some other mechanisms of negative interactions have, however, been discovered. The lowering of amino acid levels in leaves caused by root feeders can negatively affect shoot feeders (Bezemer et al. 2005) while the decrease of plant productivity by shoot feeders can have adverse effects on root feeders (Masters et al. 1993, Moran and Whitham 1990). It is furthermore noteworthy that in some of the tested systems above- and belowground herbivores did not affect each other (Staley et al. 2008), or only did so under particular levels of drought stress (Staley et al. 2007, Staley et al. 2008). The pulsed stress hypothesis (Huberty and Denno 2004), a modification of the plant stress hypothesis, states that sap feeders can only benefit from water stress induced increases in available nitrogen when in between bouts of stress turgor is allowed to recover. In addition, specialist herbivores can be well adapted to cope with certain chemical defence traits, resulting in more pronounced effects of factors such as leaf age, water content and mechanical defences on their performance (Travers-Martin and Müller 2008).

Competition between the above- and belowground herbivore could be observed under controlled conditions in the laboratory. Since aphids drastically reduced root biomass, this might explain their negative effect on the density of root-feeding nematodes. Nematodes on the other hand did not affect plant biomass, implying that their adverse effect on aphids is qualitative rather than quantitative. Interestingly, Bezemer et al. (2005) found that nematode inoculation of roots of the grasses *Agrostis capillaris* and *Anthoxanthum odoratum* reduced the performance of *Rhopalosiphum padi* aphids on the leaves. In this study the effect could be

attributed to a reduction of foliar nitrogen and amino acid concentrations. Given the relatedness between their study organisms and ours, it is not unlikely that our result was caused by a similar change in primary plant compounds.

In a natural ecosystem however, plants differ in various characteristics like size, age, spatial context, root/shoot ratio and state of turgor. Moreover, if the interactions are driven by a combination of water and nitrogen availability, it can be expected that these interactions will be hard to detect in a natural coastal dune system. The amount of water and nitrogen a plant can offer to a herbivore will be dependent on inherent features of that plant, such as root and shoot vitality, biomass, and plant age. These features will in turn be dependent on a plethora of interacting biotic and abiotic environmental factors, such as competition with other plants or position with respect to the ground water level. Of these factors, other herbivores might be of varying importance. We hypothesise that this caused the apparent lack of relationships between above- and belowground herbivores in the field and the divergent links with plant quality.

Evidently, our correlative field study does not allow the detection of plant-mediated mechanisms driving herbivore performance. The highly significant positive correlation of leaf vitality and negative correlation of plant height with numbers of aphids suggest that *S. rufula* has a strong preference for young growing shoots, which are very vital and have not reached mature size yet. In this sense, *S. rufula* is a flush feeder rather than a senescence feeder, potentially taking advantage of the increased flow of available nitrogen towards the inner expanding leaf (Coley et al. 2006). The highly significant positive correlation between root density and this species' abundance emphasises the potential additional role of water availability in a generally drought stressed environment such as sand dunes. The pulsed stress hypothesis was verified for sap-feeding insects (Huberty and Denno 2004), and our data on aphids suggest a similar dependency on both high turgor and increased levels of soluble nitrogen, albeit in young, growing plants rather than senescing ones. The positive correlation between root vitality and numbers of endoparasitic migratory nematodes suggests that these nematodes are also favoured by vital plants. In this case they could be regarded as flush feeders at the root scale. This could also explain the negative correlation with root density if nematodes choose plants with a less

dense root system that is growing towards the maximum density and is allocating elevated amounts of soluble nitrogen towards expanding root tips. The negative correlation between nematode numbers and root weight in the controlled experiment corresponds with this observation. Another possible mechanism is that nematodes have an adverse effect on root development, which in the microcosm is subordinate to the negative effect of aphids on root growth. This second option is, however, unlikely given the fact that the effect of nematode addition on root weight was highly insignificant ($p = 0.7$). The average root weight of plants that received nematodes (0.0308 g) was actually higher than that of control plants without nematodes (0.0284 g). Since these nematodes are highly mobile, continuously moving within and between roots, it is not surprising that they are able to reach the desired vital parts. Finally, if cyst forming nematodes are structured bottom–up by the root system, and more cysts are found on less vital roots, this could provide a belowground extension of the plant stress hypothesis mentioned earlier (White 1969). However, cysts could also have accumulated in the soil through time, leading to higher local densities around the root system of degenerating *A. arenaria* stands at sites with decreased sand accumulation.

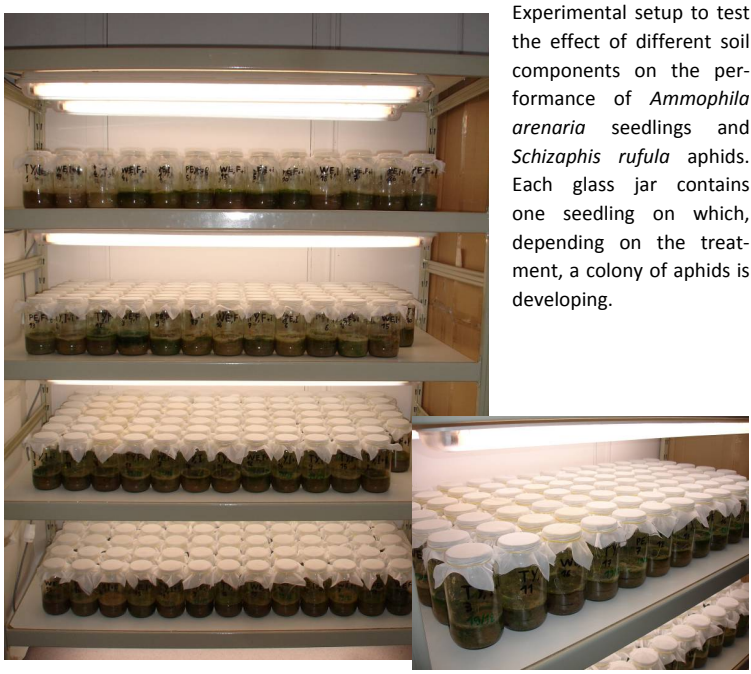
In the controlled experiment the used plants were to a high degree similar in terms of the above-discussed characteristics such as vitality, size and water availability. This demonstrates that interactions between nematodes and aphids are possible when minimising the other sources of environmental variation. A lack of proof of those same interactions in the field hence does not imply that these interactions are not existent, but rather that their result gets overruled by other factors with more pronounced effects on herbivore population dynamics.

A large body of literature documents on the various effects of aboveground herbivores on their belowground counterparts and vice versa. In the case of aphids and plant-parasitic nematodes on *A. arenaria*, clear negative effects in both directions could be observed under standardised conditions. However, no significant correlations between abundances of these two groups of organisms could be detected in the field. The specialist aphid *S. rufula* seems to be a flush feeder on *A. arenaria*, choosing young vital shoots with a dense root system. Migratory endoparasitic nematodes reached their highest abundance on less dense and more vital roots, suggesting that they are flush feeders at the root scale. Cyst densities were

highest on roots with a low vitality. It seems therefore that in this study system variables related to plant age, quality and water content are most important in structuring both above- and belowground herbivore populations in the field. Compared to other environmental factors, the effect of herbivores on these or other plant traits is not sufficient to induce a detectable effect on other herbivores at the other side of the soil surface.

3 Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics*

Martijn L. Vandegehuchte, Eduardo de la Peña and Dries Bonte



Experimental setup to test the effect of different soil components on the performance of *Ammophila arenaria* seedlings and *Schizaphis rufula* aphids. Each glass jar contains one seedling on which, depending on the treatment, a colony of aphids is developing.

* Adapted from: Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics. - PLoS ONE 5: e11174.

3.1 Abstract

Background: Plants are affected by several aspects of the soil, which have the potential to exert cascading effects on the performance of herbivorous insects. The effects of biotic and abiotic soil characteristics have, however, mostly been investigated in isolation, leaving their relative importance largely unexplored. Such is the case for the dune grass *Ammophila,* whose decline under decreasing sand accretion is argued to be caused by either biotic or abiotic soil properties.

Methodology/Principal Findings: By manipulating dune soils from three different regions, we decoupled the contributions of region, the abiotic and biotic soil component to the variation in characteristics of *Ammophila arenaria* seedlings and *Schizaphis rufula* aphid populations. Root mass fraction and total dry biomass of plants were affected by soil biota, although the latter effect was not consistent across regions. None of the measured plant properties were significantly affected by the abiotic soil component. Aphid population characteristics all differed between regions, irrespective of whether soil biota were present or absent. Hence these effects were due to differences in abiotic soil properties between regions. Although several chemical properties of the soil mixtures were measured, none of these were consistent with results for plant or aphid traits.

Conclusions/Significance: Plants were affected more strongly by soil biota than by abiotic soil properties, whereas the opposite was true for aphids. Our results thus demonstrate that the relative importance of the abiotic and biotic component of soils can differ for plants and their herbivores. The fact that not all effects of soil properties could be detected across regions moreover emphasises the need for spatial replication in order to make sound conclusions about the generality of aboveground-belowground interactions.

3.2 Introduction

Plants are heavily affected by both the abiotic and biotic properties of the soil in which they are rooted. Abiotic properties include the availability of nutrients and water, which are necessary for plant growth. Soil biota comprise mutualists as well as antagonists, exerting positive or negative effects on plant growth respectively.

These effects of soil properties on the plant can further affect leaf herbivores. Firstly, herbivores are generally limited by the nutrients they can obtain from plants, some of which these plants extract from soil (Rowntree et al. 2010), such as nitrogen (White 1978, 1984) and phosphorus (Huberty and Denno 2006). Secondly, soil biota can exert positive, negative or neutral effects on leaf herbivores, through a variety of mechanisms (reviewed in Blossey and Hunt-Joshi 2003, Hartley and Gange 2009, Koricheva et al. 2009, van der Putten et al. 2001, Vandegehuchte et al. 2010a, Wardle et al. 2004)). Positive effects can for instance arise when soil biota improve the nutritional quality of leaves (Borowicz 1997, Gange et al. 1999, Masters 1995), or damage production sites of defence molecules in the roots (Kaplan et al. 2008). Most negative effects of soil biota on leaf herbivores have been attributed to the systemic induction of chemical defences that spread from roots to shoots (Bezemer and Van Dam 2005), although root feeders can also cause a lowering of amino acid levels in leaves (Bezemer et al. 2005).

Although both biotic and abiotic soil components clearly have the potential to affect the performance of plants and their herbivores, their relative contribution to these effects has rarely been addressed. In a cross-inoculation experiment of soil biota, Joosten et al. (2009) demonstrated that both soil-borne microorganisms and the type of sterile soil affected dry mass, shoot/root ratio and the total amount and composition of pyrrolizidine alkaloids in *Jacobaea vulgaris*. Since these compounds are toxic to generalist herbivores, but preferred by specialists, it is concluded that both the abiotic and biotic soil component have the potential to affect herbivores of *J. vulgaris*. To our knowledge only a few studies have explicitly tested the combined effect of abiotic and biotic soil components on aboveground herbivores. Haase et al. (2008) investigated the interactions between collembolans (*Folsomia candida*) and aphids (*Rhopalosiphum padi*) on the

grass *Poa annua* under different levels of nutrient availability. They demonstrated that collembolans strongly increased aphid numbers at low and moderate nutrient availability, while this effect was much weaker at high nutrient availability. It has furthermore been demonstrated that the effect of mycorrhizal fungi on the performance of an insect can depend on the amount of nutrients in the soil (Koricheva et al. 2009), especially P (Borowicz 1997, Gange et al. 1999) and N (Gange and Nice 1997). The use of artificial fertiliser and the addition of large quantities of soil organisms in most of these studies raise the question whether these findings can be extrapolated to the field. Moreover, none of these studies accounted for the potential spatial variation in nutrient availability and/or soil organism density and identity one might expect to encounter in natural ecosystems.

One of the best-studied and most debated cases of negative plant-soil feedback is that of *Ammophila* species in the early succession of coastal dune vegetation. Both the North American *A. breviligulata* and the European *A. arenaria* exhibit strongly suppressed growth as sand accretion ceases, making way for later successional plant species. Therefore, vigorous stands of *Ammophila* are found in foredunes and large, dynamic inland dunes with sufficient sand-drift, while stands in stabilised dunes, often at the inner dune edge, occur as degenerate relics. This phenomenon of loss of vigour under conditions of stabilisation has been coined 'the *Ammophila* problem' by Marshall (1965). In a study of the dunes at Braunton Burrows, Willis et al. (1959a, b) hypothesised that the vigour of *A. arenaria* was due to the observed higher availability of several nutrients in the dynamic foredunes compared to the more stabilised main dunes (see Table S3.1). This was attributed to the exposure to salt spray and sand blown freshly from the beach, supposedly rich in nutrients because of the incorporation of shoreline debris. Other early work proposed interspecific competition with other plant species (Eldred and Maun 1982, Huiskes 1979) and lack of nutrients because of an inefficient replacement of old roots (Marshall 1965, Willis 1965) as explanations for the problem. However, further study on *A. arenaria* revealed that the accumulation of biotic soil factors in stabilised soils was responsible for reduced growth (Brinkman et al. 2005c, van der Putten et al. 1988, van der Putten et al. 1993). The windblown sand would thus serve as a temporary enemy-free space for the plant to root in, this is the so-called 'escape hypothesis'. Continued investigation along this line led to the

conclusion that both plant-parasitic nematodes and pathogenic fungi might be the causing agents of the observed decline (De Rooij - Van der Goes 1995, van der Putten and van der Stoel 1998, van der Stoel et al. 2002). The effect of antagonistic nematodes on plant performance was shown to be mitigated by the positive influence of mycorrhizae (de la Peña et al. 2006, Little and Maun 1996) and endophytic fungi (Hol et al. 2007). The outcompeting of harmful nematodes by less detrimental nematode species furthermore proved to be beneficial for the plant (Brinkman et al. 2005a, b), but see Brinkman et al. (2004). Moreover, it was shown that different species of root-feeding nematodes can be controlled in specific ways by soil microorganisms, other nematodes and microarthropods (Piskiewicz et al. 2008, 2009). Although these studies point at the complex nature of the interactions between different soil biota involved in this system, their net effect seems to be negative, indicating that mutualists are generally not able to overcome the effects of antagonists (Piskiewicz et al. 2009, van der Putten et al. 1993). In recent years, the original hypothesis of root efficiency of nutrient uptake has revived (Boudreau and Houle 2001). A study by Kooijman and Besse (2002) furthermore attributes the expansion of *A. arenaria* in lime- and iron-poor dunes to the relatively higher availability of P when it is not sequestered into iron and aluminium phosphates. In the absence of P-limitation, the ecosystem thus becomes N-limited. This gives *A. arenaria* an advantage because of the relatively efficient recycling of N from its own litter. Moreover, the system then becomes sensitive to atmospheric N-deposition, which further enables the grass to expand.

In a previous study, we demonstrated that the natural root-feeding nematode community of *A. arenaria* was capable of reducing the population growth of the specialist aphid *Schizaphis rufula* under laboratory conditions. Yet no correlation between nematode and aphid abundances could be detected in a field survey conducted at six spatially separated sites. Both nematode and aphid abundances could however be explained by several plant characteristics (Vandegehuchte et al. 2010a). This suggests that the effect of nematodes on aphids might be overruled in the field by other environmental factors with stronger effects on those plant traits that determine the aphids' performance.

Given this multitude of studies using *A. arenaria* to investigate plant-soil interactions, we chose this species as a model system. We inoculated sterile

soils from dynamic and stabilised dunes with an inoculum of non-sterile soil from either location and used a fully sterile soil as control. We were primarily interested in the net effect of the complex community of interacting soil biota as it occurs in nature. Therefore, we preferred a whole soil inoculum over the addition of certain species or groups of soil organisms, even though such a 'black box' approach does not enable to identify which organisms contribute to observed effects. On these soils we grew seedlings of *A. arenaria*, on half of which we let a population of *S. rufula* develop. This setup was replicated with soils from three distinct regions along the Belgian coast, to assess the generality of potential results across large spatial scales (Newton et al. 2009). This fully-crossed experiment allowed us to specifically address the following questions: 1) what is the relative importance of biotic and abiotic soil properties regarding their effect on aphid population dynamics? 2) are potential effects consistent across spatially separated dune systems, or are there regional differences? 3) if abiotic soil properties are important, which ones would make plausible candidates to explain the observed effects? Based on the evidence that soil-borne organisms are involved in the *Ammophila* problem, we hypothesise that plants should grow better on sterile soils compared to soils with naturally occurring biota. On the other hand, if this phenomenon is to some extent caused by an increased availability of nutrients in dynamic dune soils, plants should perform better on dynamic than on stabilised dune soils, irrespective of their biotic state. We hypothesise that the lower availability of nutrients should cause the performance of aphids on plants grown in sterile soils to be lower for stabilised than for dynamic dune soils. Because the accumulation of plant pathogens in stabilised dune soils is expected to result in reduced plant growth, we hypothesise that aphids should perform worse on soils inoculated with stabilised dune biota. Given the usual variability in the distribution of soil nutrients and organisms, we expect the magnitude of these effects to differ between regions. We hypothesise that the availability of nitrogen and phosphorus in soils should match the performance of aphids better than that of other abiotic elements.

3.3 Material and methods

3.3.1 Experimental setup

Soil was collected from three different regions at the coast on 5 November 2008: nature reserve Westhoek at De Panne (Belgium), nature reserve Ter Yde at Oostduinkerke (Belgium) and Le Perroquet at Bray-Dunes (France). In each region soil was collected from two sites; one situated in dynamic dunes with high amounts of sand accretion where *A. arenaria* grows very vigorously and one situated at the inner dune edge, where conditions are more stabilised and the plants decline in vigour. These more stabilised sites were all located in places with considerable areas of bare sand where *A. arenaria*, although degenerating, still occurs as large tussocks. Dunes where very degenerate relics of *A. arenaria* are present among other, later successional vegetation were not considered in this study. All these dune areas are spatially separated (distances between sites from different regions ranging from 2.1 to 12.6 km). At each site, a composed sample of soil was taken, collected from underneath different stands of *A. arenaria,* comprising a mixture of upper root zone soil and freshly deposited soil from above the root zone. In the laboratory each sample of soil was divided into two parts, one of which was sterilised by autoclaving for 1 hour at 120°C and 1 atm. For each site three types of soil were prepared: fully sterile soil, sterile soil with an inoculum of non-sterile soil from the same site and sterile soil with an inoculum of non-sterile soil from the other site within the same region. So for each region the combinations were: D, D+s , D+d, S, S+d, S+s, with D = sterile dynamic dune soil, S = sterile stabilised dune soil, d = unsterilised dynamic dune soil inoculum, s = unsterilised stabilised dune soil inoculum. The inoculum comprised 21 volume percent of the soil mixture, which did not affect any of the purely abiotic properties of the soil mixture (see Text S3.2, Fig. S3.6, Fig. S3.7, Fig. S3.8, Table S3.3 and Table S3.4 in 9. Supplementary Information). This way the effect of the soil's abiotic and biotic component could be decoupled.

Seeds of *A. arenaria* were collected from the nature reserve Westhoek from a single stand. Seeds were surface sterilised by submersing in 4% household bleach solution for 5 minutes, rinsing 10 times with demineralised

water, submersing in 10% ethanol for 5 minutes and rinsing another 10 times with demineralised water (adapted from Sauer and Burroughs 1986). This sterilisation method effectively eliminates horizontally transmitted fungi that could otherwise colonise the young seedling. No endophytic fungi were detected in the endosperm of seeds from the sampled plant population (M. Omacini and E. de la Peña, unpublished result). Seeds were subsequently germinated at a light regime of 9/15 hours dark/light in plastic 1 l pots filled with 190 cm^3 of commercial white sand that was autoclaved for 1 hour at 120 °C and 1 atm. The sand was saturated with demineralised water. Plastic foil that covered the pots was perforated to allow of enough ventilation. Moisture level was reset to near saturation 3 times a week.

For each unique soil type, twenty replicate 1062 ml glass jars were each filled with 300 ml of the treatment soil. Each jar received one six week old seedling, and from then on water was added twice a week, alternately with and without fertiliser (Compo NPK 16-9-20, 1 g l^{-1} tap water). The opening of each jar was sealed with a piece of fine-meshed gauze.

After having grown on the different soils for 5 weeks, ten plants of each treatment soil type received a single first instar nymph of the specialist aphid *S. rufula*, that was allowed to become adult and reproduce parthenogenetically. Aphids were counted daily. When a substantial decrease in aphid numbers occurred, the plant was harvested and all remaining aphids were transferred to 70% ethanol. The plant was uprooted and root and shoot were weighed fresh. They were subsequently oven dried at 65°C overnight and weighed again.

Plants that did not receive aphids acted as a control. They were uprooted 12 weeks after transplantation to the different soil types. Root and shoot were weighed fresh before they were oven dried at 65°C overnight to determine dry weights. Of each soil type three replicates were selected for soil analysis. The following soil characteristics were determined: percentage moisture (g water per 100 g of fresh soil), NO_3-N (mg kg^{-1} of dry soil), NH_4-N (mg kg^{-1} of dry soil), plant available P (mg kg^{-1}, Olsen method), pH-KCl, percentage organic matter per dry soil and percentage $CaCO_3$ (see Text S3.2 for methodology).

3.3.2 Analyses

The effects of the soil treatments on plant performance were tested within the control group of plants that did not receive aphids, because the effect of aphids on plants can potentially interact with the effects of the soil treatments. Tested plant variables were total dry weight, which was highly correlated with shoot dry weight, root dry weight, the root proportion of total dry weight and relative water content of the shoot, which was highly correlated with the relative water content of the entire plant. These variables were chosen because they represent measures of total biomass produced, the relative allocation of biomass to roots and shoots, and the vitality of the plant tissue.

Several aphid population parameters were tested in function of the soil treatments. The number of days between introduction of the first instar and the appearance of the first offspring was used as an approximation of generation time. The number of aphids at the population peak equates the maximum population size a plant can sustain. For each population, an exponential growth curve was fitted through the aphid abundances from day one until the day of population peak. The growth constant k of the curve $N = N_0.e^{kt}$ served as a measure of population growth rate.

To determine which treatments or interactions between treatments significantly affected plant and aphid characteristics, a permutational 3x2x3 ANOVA was performed for each dependent variable. The treatment 'region' was composed of levels 'Westhoek' (WE), 'Ter Yde' (TY) and 'Perroquet' (PE). The treatment 'soil' refers to the sterile part of each treatment soil with levels 'dynamic dune' (D) and 'stabilised dune' (S). The treatment 'inoculum' refers to the non-sterile soil inoculum with levels 'none' (/), 'dynamic dune' (d) and 'stabilised dune' (s). Tests were based on type III sums of squares and 99,999 permutations of the residuals under a reduced model. A backward stepwise pooling of non significant terms ($p > 0.05$) was performed to obtain robust p-values in the final model. If the final model happened to retain only one predictor variable, the test was repeated with unrestricted permutation of raw data, which provides an exact test for the one-way case (Anderson et al. 2008). Homogeneity of variances was tested with a permutational Levene's test using 99,999 permutations. In the case of unequal variances, a non-parametric test was performed by applying the above-described

permutational ANOVA to the ranks of the original data. Pairwise differences between levels of significant factors were tested for each final model by means of a permutational t-test with 99,999 permutations. When the number of unique permutations was lower than 100, Monte Carlo sampling was used to obtain reliable p-values.

Correlations between each aphid population parameter and the different plant characteristics were determined across all replicas that had received aphids. The plant feature that correlated best with each dependent variable was incorporated as a continuous covariate into the above-described ANOVA models. By comparing models with and without covariate, effects of soil treatments on aphids that are due to differences in plant growth or vitality could be decoupled from additional plant physiological effects.

Out of 180 introduced aphid nymphs, 16 died before reproducing and these replicas were omitted from all analyses. Seedlings that died in the early stages after transplantation (77 out of 360) were replaced, but since this replacement affected the measured characteristics, only the initial plants were retained in all analyses. There were no significant differences in seedling mortality between regions, soil types, or inocula (Vandegehuchte et al. unpub. data).

To determine whether the soil properties of our treatment soils significantly differed according to region, soil, inoculum, or an interaction, a permutational 3x2x3 ANOVA with a backward selection procedure was performed for each soil parameter in a similar way as described above.

3.4 Results

3.4.1 Plants

Plant dry weight differed significantly according to an interaction between region and inoculum (pseudo-$F_{4,93}$ = 3.7674, p = 0.0068, Fig. 3.1a), with the main effect of inoculum being significant (pseudo-$F_{2,93}$ = 3.4922, p = 0.0336) and the main effect of region being marginally significant (pseudo-

$F_{2,93}$ = 2.7043, p = 0.0711). The type of sterile soil did not affect plant dry weight. Pairwise comparisons revealed that on soils from Westhoek and Le Perroquet there was no effect of soil biota on total dry weight, regardless of their origin. On Ter Yde soils, however, dry weight clearly increased when no soil biota were inoculated (Table S3.5). Root dry weight was not significantly affected by any of the treatments.

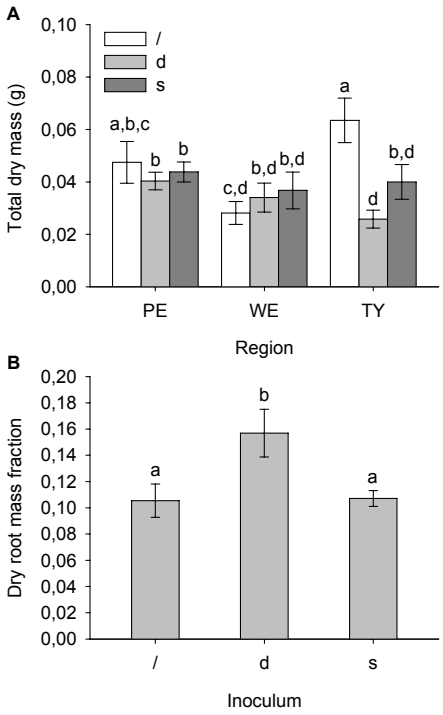


Figure 3.1 Effect of different soil treatments on characteristics of *A. arenaria* seedlings (mean + SE). A) Effect of region and soil inoculum on total dry mass of the plant. B) Effect of soil inoculum on the root fraction of the total plant dry mass. Significant pairwise differences are indicated by different letters above the bars ($p < 0.05$). Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde. Inoculum - /: no inoculum, d: dynamic dune biota, s: stabilised dune biota.

Although for plant dry weight the Levene's test was significant (p = 0.0269), the F-test of ANOVA is very robust against unequal variances, and a significance level of 0.01 has been suggested for homogeneity tests prior to ANOVA (McGuinness 2002, Neter et al. 1996). We therefore consider the presented results valid. The non-parametric test moreover confirmed the significant interaction between region and inoculum (pseudo-$F_{4,93}$ = 3.2103, p = 0.0165).

Homogeneity of variances could not be confirmed for the relative water content of the shoot (Levene's test, p = 0.001), and according to the non-parametric test, none of the treatments had a significant effect.

The root fraction of dry weight was only affected by soil inoculum (pseudo-$F_{2,99}$ = 4.8511 , p = 0.0025), as a larger proportion of the total dry weight was allocated to roots in plants grown on soils inoculated with biota from dynamic dunes (Fig. 3.1b). This effect was irrespective of whether the sterile soil part originated from dynamic or stabilised dunes and of the region along the coast. Levene's test (p = 0.3768) did not reject the assumption of homogeneity of variances. Summarising these results, it can be concluded that *A. arenaria* seedlings were most affected by soil biota.

3.4.2 Aphids

The plant feature that correlated best with aphid maximum density was the fresh weight of the shoot (Pearson's r = 0.74221). Total fresh weight correlated best with generation time (Pearson's r = 0.49349), while the exponential growth constant correlated best with relative water content of the shoot (Pearson's r = 0.32240).

Results of the ANOVA demonstrate a significant effect on maximum aphid density only of region (pseudo-$F_{2,161}$ = 7.174, p = 0.001, Fig. 3.2a). Modelling shoot fresh weight as a covariate (pseudo-$F_{1,160}$ = 194.88, p = 0.00001) still resulted in a significant effect of region (pseudo-$F_{2,160}$ = 6.7923, p = 0.0008). This indicates that regional soil effects do not only operate through changes in plant shoot weight, but also through additional mechanisms. Again only region was retained as a significant factor affecting aphid generation time, both in the models without (pseudo-$F_{2,161}$ = 3.1168, p = 0.0356) and with (pseudo-$F_{2,160}$ = 3.6218, p = 0.0252) total fresh weight (pseudo-$F_{1,160}$ = 52.845, p = 0.00001) as a covariate. No significant pairwise differences could be detected, but the differences between Le Perroquet and Westhoek (p = 0.0554) and between Westhoek and Ter Yde (p = 0.0601) were almost significant (Fig. 3.2b, Table S3.5). Region turned out to be the only factor significantly affecting the aphids' population growth constant (pseudo-$F_{2,161}$ = 3.1898, p = 0.0427). A similar result (pseudo-$F_{2,160}$ = 3.2488, p = 0.0403) was obtained when the relative water content of the shoot was

modelled as a covariate (pseudo-$F_{2,160}$ = 18.729, p = 0.00007). Aphid populations on plants from Ter Yde were characterised by stronger exponential growth, short generation times and larger maximum population sizes (Fig. 3.2). In all final models for aphid population parameters, variances did not significantly differ between groups (Levene's test, p > 0.05).

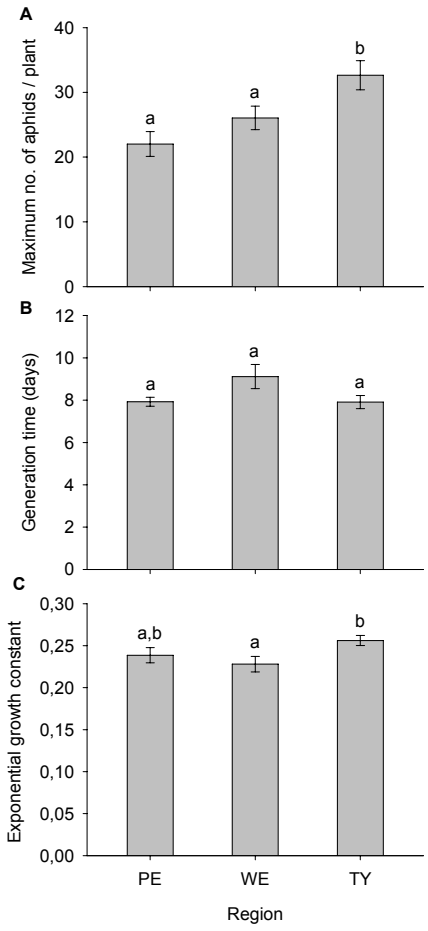


Figure 3.2 Effect of soil region of origin on *S. rufula* aphid population dynamics (mean + SE). A) Effect of region on the maximum number of aphids. B) Effect of region on the generation time of the first aphid. C) Effect of region on the growth constant k of the exponential growth curve. Significant pairwise differences are indicated by different letters above the bars (p < 0.05). Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde.

In contrast to the plants they lived on, *S. rufula* aphids seemed not to be affected by the presence or nature of the biota in the soil. On the other hand, all tested population parameters significantly differed between regions where soil was collected. Since no interaction with inoculum was significant, some abiotic difference between the three dune regions must have been responsible for the observed differences. Therefore, we conclude that aphids are more affected by the abiotic properties of the soil than by the biotic soil component.

3.4.3 Soil

Although most of the measured soil parameters differed significantly according to one or more treatment factors, none of the observed patterns suggested a logical causal link to the effects of the soil treatments on plant and/or aphid characteristics. Details of the differences in soil parameters between treatment soil mixtures are given in Text S3.2, Fig. S3.6, Fig. S3.7, Fig. S3.8, Table S3.3 and Table S3.4.

3.5 Discussion

3.5.1 Plants

The growth of *A. arenaria* seedlings was clearly more affected by the biotic than by the abiotic component of the soil they were planted in. There was no effect of the origin of the sterile soil part – dynamic or stabilised dune – on any of the tested plant characteristics. However, the effects of soil biota on biomass production were not consistent across regions.

We collected soils from dynamic dunes in winter, the period when most sand accretion occurs and plants have not yet developed roots into the newest layer (van der Stoel et al. 2002). Therefore the nutrients present in the fresh top layer of sand could not yet have been depleted by plants in the field. Since we could not detect any difference in plant performance between sterile soils from dynamic and stabilised dunes, the hypothesis that the

decline of *A. arenaria* is due to the decreased availability of nutrients could not be confirmed in our study. The observation that biomass production was reduced by soil-borne biota from the rhizosphere of vital as well as degenerated plants is in accordance with previous work (van der Putten et al. 1993, van der Putten and van der Stoel 1998). However, this result was only confirmed for soils from Ter Yde. One explanation is that only Ter Yde soils contain organisms able to cause observable reductions in biomass within a limited amount of time, either because they are more pathogenic or because they have a higher proliferation rate. For example, some studies have demonstrated that, depending on the conditions, such as species combinations and densities, certain root-feeding nematode species do not always significantly affect the biomass of *A. arenaria* (Brinkman et al. 2005a, b, de la Peña et al. 2006, de la Peña et al. 2008). Moreover, in a study using a comparable experimental setup (Vandegehuchte et al. 2010a), we found no effect of the extracted nematode community of Westhoek dune soils on either root or shoot biomass of *A. arenaria* seedlings. A second explanation might be that soils from Ter Yde contain relatively less organisms that are beneficial to the plant, such as mycorrhizae, or able to control plant antagonists, such as microbes and microarthropods. On the other hand, plants on sterile soil from Ter Yde produced significantly more biomass than those grown on sterile soil from Westhoek. This implies that some other, abiotic soil factor is limiting plant growth on Westhoek soils. Our experimental setup does not allow determining whether or not this abiotic effect is overruling a potential effect of soil biota as observed on Ter Yde soils. None of the measured soil properties was, however, indicative of a difference between Ter Yde and the other regions that could explain the observed pattern (see Text S3.2, Fig. S3.6, Fig. S3.7, Fig. S3.8, Table S3.3 and Table S3.4).

The proportion between root and shoot mass was not affected by the origin of the sterilised soil part, while plants inoculated with dynamic dune biota had a higher relative amount of roots. Note that the absolute root weight was not significantly higher for plants inoculated with dynamic dune biota, so this effect was largely due to a lower shoot biomass of these plants. Since this effect was equal across the three regions, it seems that dynamic dunes, where *A. arenaria* thrives best, generally harbour soil organisms that affect the allocation of resources to different plant parts. Several studies

have suggested that the windblown sand enables the plant to replace its old roots, hence increasing the ability to extract the necessary nutrients (Marshall 1965, Willis 1965). Our results demonstrate that even in young seedlings, the relative allocation of biomass to roots is increased in the presence of soil biota specific for the dune stages where sand accretion occurs. Interestingly, biota of later successional soils did not exert this effect. Relative root mass of plants grown on soil inoculated with stabilised dune biota was equal to that of plants grown on sterile soil. One explanation might be that the soil sampled at dynamic dune sites contains a lower total abundance of root antagonists than soil from stabilised dunes. There is evidence that at low densities, root-feeding nematodes can cause an enhanced translocation of photosynthate to roots, ultimately leading to a relative increase in root biomass (Bardgett et al. 1999, Poll et al. 2007). The positive effect on relative root mass of soil biota, at the low abundances typical of dynamic dunes, might thus have disappeared as densities increased under stabilising conditions. A second possibility is that a difference in the identity, rather than the abundance, between the soil biota from dynamic and stabilised dunes caused the observed difference in relative root mass. In a study on the nematode community along a sand dune succession in Scotland, Wall et al. (2002) demonstrated a shift in species composition between foredunes on the one hand and yellow dunes and grey dunes on the other. They further found that both total and plant-feeding nematode abundance increased along the successional gradient. This indicates that both proposed mechanisms, altered densities and species composition, could be operating synergistically. Both abundance and diversity of arbuscular mycorrhizal fungi were moreover found to be higher in isolates from vital than from degenerate stands of *A. arenaria* in coastal dunes of the Netherlands (Kowalchuk et al. 2002). In dynamic dunes, the lower net effect of plant antagonists might thus be further mitigated by the higher abundance of these fungi, since they are mutualistic to the plant.

3.5.2 Aphids

Contrary to our hypotheses, none of the tested aphid population characteristics differed between soils or inocula from dynamic and stabilised dunes. However, differences were detected in aphid population size, exponential growth constant and individual generation time between soils

from the three regions along the coast. Aphids displayed the shortest generation times, steepest exponential growth and largest final population sizes on plants grown on soils from Ter Yde. These results did not change after correcting for the most significant correlations with measured plant characteristics, indicating that they are due to changes in the host plant of a fine-scale physiological nature. If these effects were due to differences in soil biota, they would have been detected for inoculated soils only, leading to a region x inoculum interaction. The observation that differences were independent of the biotic state of the soil, provides indirect proof that they must have been due to differences in one or more abiotic properties of the soils from the three regions. Although significant main effects of region on soil pH, % organic matter, % $CaCO_3$ and plant available P were detected (see Text S3.2, Fig. S3.7, Fig. S3.8, Table S3.3 and Table S3.4), the inspection of significant interaction terms with soil and inoculum demonstrated that none of these soil parameters would logically explain the observed differences in aphid population properties across regions. For example, the higher amount of plant available P in Ter Yde soils might seem to explain why aphids performed better on plants grown on soils from this region. However, when regressing the maximum number of aphids on amount of P within each region (data not shown), contrasting results are obtained for each region, indicating that the relation between P and aphid population size does not hold. Although N would make a plausible predictor of aphid performance, no differences in NO_3-N or NH_4-N content could be detected between soils from different regions. It thus seems that in our system, aphids were not affected by any of the measured soil characteristics, not even by N or P availability, although these are the two elements generally considered to be limiting for herbivorous insects (Huberty and Denno 2006, White 1984). It is possible that differences in the content of some other (micro)nutrient or in some physical property of the soil, e.g. pore size, caused the observed effects of region on aphids, especially given the subtle nature of the plant features that caused these effects.

The observation that soil biota did not affect aphid performance, although they did affect plant performance to some extent, is not in line with the bulk of literature documenting on the interactions between below- and aboveground biota (Blossey and Hunt-Joshi 2003, Koricheva et al. 2009, van der Putten et al. 2001, Vandegehuchte et al. 2010a, Wardle et al. 2004).

However, some effects of soil biota on aboveground herbivores have been shown to only become apparent under particular levels of drought (Staley et al. 2007, Staley et al. 2008). Since our plants were watered ad libitum, soil biota might have been unable to impose stress on plants that would elicit a response of the aphids. Interestingly, in a similar laboratory setup, we have previously demonstrated a negative effect of the community of root-feeding nematodes on the population size of *S. rufula* (Vandegehuchte et al. 2010a). However, the nematodes in the cited study were obtained by extraction from large quantities of roots, and subsequently concentrated to rather high densities before inoculation. Here we chose to use a complete soil inoculum, in order to address the more general question of the relative importance of the biotic soil component as such. Therefore, the volume of non-sterile soil had to be small compared to the sterilised bulk part, in order not to quantitatively change the abiotic properties of the inoculated soils. The number of nematodes in such a small soil volume, especially root-feeding ones, is probably too low to cause a similar negative effect. However, the densities of biota applied here probably reflect the field situation more accurately. This is further supported by the lack of correlation between root-feeding nematode and aphid abundances in the field survey of the cited study.

3.5.3 Implications of spatial variation

The fact that all the aphid population variables differed according to the soil's region of origin, but not according to the successional state of the dune soil or the biota present in that soil, emphasises the importance of replication at larger spatial scales. If our study had only focused on one particular dune system, we would probably have concluded that no single aspect of the soil affected the development of aphid populations. As for the plant characteristics, the total dry biomass of plants only differed according to soil biota for soils from the Ter Yde dune area. By replicating our setup across dune areas, it became clear that this effect of biota is not a general phenomenon. The positive effect of dynamic dune biota on relative root biomass on the other hand turned out to hold true for each of the three dune areas, thereby proving the generality of this relationship. The specificity of some effects of biota for certain locations was to be expected since the spatial distribution of soil organisms is generally heterogeneous. This implies

that at different locations, different species assemblages of soil biota occur, as for example demonstrated for root-feeding nematode species of the genus *Pratylenchus* associated with *A. arenaria* (de la Peña et al. 2007). Given this variability in the universality and/or magnitude of the observed effects across spatially separated systems, spatial replication in future studies of above-belowground interactions, and of ecology in general, is needed to provide a better understanding of the generality of the ongoing processes.

3.5.4 Conclusions

In order to unravel the relative effects of the abiotic and biotic components of soil on plant and insect herbivore performance, we chose *A. arenaria* as a model species because of its specific ecology. The decline of this species under decreasing sand dynamics has been attributed either to soil biota or to abiotic soil properties in several studies over the past decades. Our study did not yield convincing evidence of abiotic soil effects on plant performance, while soil biota did affect plant traits, in accordance with the escape hypothesis, although not all effects were apparent in all of the investigated dune areas. Even though the potential of soil biota to affect aphid population dynamics has previously been demonstrated in this system, this study could not detect any effect of soil biota on the performance of aphids. Differences of abiotic nature between soils from the three dune areas however affected all of the tested aphid population parameters. Our results therefore suggest that the biotic soil component is more important than the abiotic component in affecting plant performance, while the opposite holds true for the insect herbivore.

4 Mycorrhizal fungi decrease *Ammophila arenaria* seedling growth and aphid population size*

Martijn L. Vandegehuchte, Roeland Cortois, Eduardo de la Peña and Dries Bonte

Schizaphis rufula feeding on a leaf of *Ammophila arenaria*.

---

* Adapted from: Vandegehuchte M. L., Cortois R., de la Pena E. and Bonte D. under review. Mycorrhizal fungi decrease *Ammophila arenaria* seedling growth and aphid population size. - Acta Oecologica.

4.1 Abstract

The majority of vascular plants develop associations with mycorrhizal fungi, in which the plant provides the fungus with carbon in exchange for limiting nutrients. Mycorrhizal induced changes in a plant's chemical defence system and to a lesser extent nutritional quality, have been shown to affect the performance of insect herbivores. Although mutualism seems to be more common, the symbiosis can turn into parasitism under certain conditions.

We investigated the effect of arbuscular mycorrhizal fungi (AMF) on the growth of *Ammophila arenaria* seedlings and population dynamics of the specialised aphid *Schizaphis rufula*, across different levels of water availability. Both root and shoot biomass were severely reduced by AMF. As young seedlings obtain most nutrients from seed reserves, the cost of providing the fungus with carbon might have exceeded the benefit of a nutrient gain. Aphids reached a significantly smaller population size on seedlings that received AMF. Aphid population size was highly correlated with shoot dry biomass. When correcting for this relationship, the effect of AMF disappeared. Moreover, aphid development time and population growth rate were not affected by AMF. Together these findings suggest that the effect of AMF on aphids was not caused by changes in nutrient availability or allelochemical levels in the plants, but by changes in leaf quantity.

Altered plant biomass hence presents a simple and relevant but hitherto overlooked mechanism by which mycorrhizal fungi can affect herbivorous insects.

4.2 Introduction

All terrestrial vascular plants harbour some species of herbivorous insects (Schoonhoven et al. 2005). Mycorrhizal associations between plants and fungi are also widespread in terrestrial ecosystems (Hodge 2000). Of the seven distinguished types, the most common association is the arbuscular mycorrhiza (AM) that is formed by approximately 70% of the world's vascular plants (Brundrett 1991, Hodge 2000). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that establish a symbiosis with a plant from which they obtain carbon. In exchange, they assist the plant with the acquisition of mineral nutrients, particularly phosphorus (Harrison 2005). Although AMF are generally considered plant mutualists, it has been demonstrated that the effects of these mycorrhizae on plant performance can also be neutral or become negative when net costs exceed net benefits (Graham and Abbott 2000, Koide 1985, Reynolds et al. 2006, Sudova and Vosatka 2008). The density of AMF at which plant benefit is maximal, might differ according to plant and fungus species identity (Gange and Ayres 1999, Klironomos 2003), soil biotic or abiotic conditions (Hoeksema et al. 2010, Johnson et al. 1997), or level of irradiant light (Johnson et al. 1997). Especially when the resources provided by AMF (predominantly phosphorus) are increased in the soil, the mutualistic interaction often changes into a parasitic one (Hoeksema et al. 2010, Johnson et al. 1997). It has furthermore been observed that AMF depress seedling growth in the first weeks following germination, because at this stage necessary resources are obtained from seed reserves, and loss of carbon to the fungus decreases allocation to photosynthetic or defence structures and growth (Johnson et al. 1997). Another important abiotic actor on the plant-mycorrhizal interaction is water availability. Plant drought resistance is known to be generally improved by AMF, often indirectly through growth enhancement which in turn is related to improved nutrient acquisition, but also through a series of direct hydraulic effects (Augé 2001).

Given this mutualism-parasitism continuum of plant-mycorrhizal interactions, it is not surprising that AMF have the potential to affect insect herbivores in equally diverse ways (Hartley and Gange 2009, Koricheva et al. 2009). Generalist chewing insects and mesophyll feeders are mostly negatively affected by mycorrhizal fungi, whereas phloem feeders and specialist chewers are usually benefited (Hartley and Gange 2009, Koricheva

et al. 2009). This suggests that mycorrhizal effects on insect herbivores are primarily due to the activation of plant defences. Chewing and sucking insects feeding on cell contents are indeed more prone to ingestion of secondary metabolites, which are commonly stored inside cells, than sucking insects feeding on phloem, which is largely void of any defensive compounds (Koricheva et al. 2009). However, some studies have demonstrated effects of AMF on arthropod herbivores through altered plant nutrient status (Bonte et al. 2010, Gange and Nice 1997, Gange et al. 2005, Goverde et al. 2000, Hoffmann et al. 2009).

The dune grass *Ammophila arenaria* (L.) Link has been used as a model species for the investigation of plant-soil feedbacks over the past two decades, because of its specific ecology. This grass only thrives in dynamic dunes with considerable sand-drifts, as growth is severely suppressed when sand accretion ceases, making way for plant species of the next stage in succession (Huiskes 1979). Experimental studies have demonstrated that soil biota, presumably root-feeding nematodes or pathogenic fungi, were the causative agent of the observed decline (Brinkman et al. 2005b, De Rooij - Van der Goes 1995, van der Putten et al. 1988, van der Putten et al. 1993, van der Putten and van der Stoel 1998, van der Stoel et al. 2002). However, harmful nematodes can be controlled to some extent by less detrimental nematode species (Brinkman et al. 2005a, b), microorganisms, microarthropods (Piskiewicz et al. 2008), endophytic fungi (Hol et al. 2007) and mycorrhizal fungi (de la Peña et al. 2006, Little and Maun 1996).

This dune grass also hosts a suite of aboveground invertebrate species, including some highly specialised herbivores (Huiskes 1979, Vandegehuchte et al. 2010b). Although root-feeding nematodes are able to reduce population growth of the specialist aphid *Schizaphis rufula* (Vandegehuchte et al. 2010a), no correlation between nematode and aphid abundances could be found under natural conditions. Moreover, no effect of a whole soil biotic inoculum could be detected on *S. rufula* performance in the laboratory (Vandegehuchte et al. 2010c). Throughout Europe, AMF are omnipresent in roots of *A. arenaria* (Rodríguez-Echeverría et al. 2008b). These AMF have the potential to benefit the performance of this grass in the presence of root herbivores (de la Peña et al. 2006). It is therefore not unlikely that AMF compensated the potential negative effect of root-feeding nematodes in the inoculum of the lab experiment and/or mitigated the effect of nematodes in

the field survey. In this paper, we aim to broaden the knowledge on above-belowground interactions within the *A. arenaria* model system, by addressing these uncertainties concerning the effects of AMF on both plant growth and aphid performance experimentally. We replicated our experiment across different levels of water availability, since water levels can affect both the interaction between AMF and the plant (Augé 2001), and the performance of insect herbivores (Huberty and Denno 2004). We hypothesise that AMF benefit plant growth, which in turn leads to increased aphid performance, and that the strength of these effects depends on the degree of water availability.

4.3 Material and methods

4.3.1 Experimental design

Seeds of *A. arenaria* were collected in the summer of 2008 from a single stand in the Flemish nature reserve Westhoek (Belgium) and stored at 4 °C. They were surface sterilised on 1 October 2009, by consecutive submersing in a 4% household bleach solution for 5 minutes, rinsing 10 times in demineralised water, submersing in 10% ethanol for 5 minutes and rinsing 10 times in demineralised water (adapted from Sauer and Burroughs 1986). This sterilisation method effectively eliminates horizontally transmitted fungi that could otherwise colonise the young seedling. No endophytic fungi were detected in the endosperm of seeds from the sampled plant population (M. Omacini and E. de la Peña, unpublished result).

The seeds were then germinated in a growth chamber under an 8/16 h dark/light regime on dune sand from the Westhoek nature reserve that was sterilised by autoclaving for 1h at 120°C, in plastic 1L pots sealed with a perforated plastic foil.

One individual two week old *A. arenaria* seedling was transplanted to each of 150 sterile plastic 1L pots containing 400 g of sterile sand from the Westhoek nature reserve. The bottom of the plastic pots was perforated and pots were placed in a plastic tray that allowed water to be administered

during the experiment. The pots were sealed with a piece of fine mesh cloth. At this stage of the experiment, all pots received equal amounts of water every 2 to 3 days. Plants remained in the growth chamber under an 8/16 h dark/light regime until the end of the experiment.

One week after transplantation, 75 of 150 plants were inoculated with a mix of spores from four *Glomus* species: *G. intraradices, G. aggregatum, G. etunicatum* and *G. mosseae*, and are further referred to as mycorrhizal plants (M). Each pot received an inoculum consisting of 0.5 g of commercial pure spore mix, containing 142 spores on average, in 35 ml of demineralised water. This amount of spores per weight of soil is at the lower end of the range observed in the field (Kowalchuk et al. 2002, Rodríguez-Echeverría et al. 2008b), which seemed appropriate given the small size of the seedlings. Moreover, in an experiment using *A. arenaria* seedlings of the same age, de la Peña et al. (2006) used a comparable amount of inoculum. The inoculum was injected into three holes around the roots of the plant. The other 75 plants received an equal amount of demineralised water and served as a non-mycorrhizal control (NM). The majority of AMF that naturally associate with *A. arenaria* belong to the genus *Glomus* (Rodríguez-Echeverría et al. 2008b), some of which have been identified as *G. intraradices* (de la Peña et al. 2006). Therefore, the effects of the used species mixture are expected to be comparable to those of the natural community.

When seedlings were four weeks old, a differential watering treatment was included in the experiment. One third of all plants received 25 ml demineralised water every 3 days (low availability, L), another third received 50 ml demineralised water every 3 days (high availability, H) and the last third received 100 ml distilled water every 6 days (intermediate availability, I). After three watering sessions, intervals between watering bouts were increased to 9 days for the I-group, to increase the difference with the H-group. After every watering session, plants were randomised in order to avoid positioning effects. Experience from previous experiments has taught that seedlings can suddenly wilt completely when the soil becomes drier. The used water levels were such that even the lowest still supported vigorous growth of the seedlings. *A. arenaria* mostly reproduces clonally in the field, and seeds can only germinate under moderately wet to wet conditions, e.g. in places where the wind has blown out the sand to the groundwater table

(Huiskes 1979). Our watering treatment thus reflects the natural range of moisture levels of habitats in which seedlings occur in the field.

When they were five weeks old, 15 out of 25 plants of each unique treatment combination (NM-L, NM-I, NM-H, M-L, M-I, M-H) received a single newborn *S. rufula* first instar, that was allowed to develop into a parthenogenetic, reproducing adult. The remaining 60 plants were not subjected to aphid herbivory. First instars were obtained from a laboratory culture originally stemming from a wild population in the Westhoek nature reserve and kept on *A. arenaria* plants, grown on sterile soil from seeds collected in the same nature reserve. From the day of introduction onward, aphids were counted daily until the population crashed and no live aphids could be detected. In short, the experimental design is a full factorial one with 3 treatments: aphid herbivory (aphids: A, no aphids, NA), inoculation with *Glomus* spp. spores (M and NM), and water availability (L: low, I: intermediate, H: high).

One of the primary symbiotic effects of AMF is an enhanced plant phosphorus acquisition (Fitter 2006), and AMF can thus influence the nutritional status of plant tissues via this mechanism (see introduction). When plants were seven weeks old, all pots received 25 ml of a modified Hoagland solution, without phosphorus. To allow for this possible effect to occur, the phosphorus concentration in the soil was kept low by not including this nutrient in the Hoagland solution.

On the 31st day after the introduction of the aphids, all populations had crashed. Plants were uprooted and the biomass of roots and shoots of the plants were measured separately. Roots were rinsed with tap water. After weighing, shoots were stored at -18 °C and roots were put in 50% ethanol until further use. Shoots were oven dried for 72 h at 50 °C and weighed again. Roots were stained with acid fuchsin according to Baker and Gowen (1996), and screened for mycorrhizal structures which verified the presence of AMF only in roots of the inoculated plants.

4.3.2 Analyses

The effects of aphid addition, mycorrhizal inoculation and drought stress on plant characteristics were tested by means of a three-way permutational analysis of variance (ANOVA), based on Euclidean distance and 99,999 permutations of residuals under a reduced model (Anderson et al. 2008). The tested dependent variables were: root fresh weight (g), shoot fresh weight (g), shoot dry weight (g), relative water content of the shoot, total fresh weight (g) and root fraction of the fresh weight.

The effects of mycorrhizal inoculation and water availability on aphid characteristics were tested by means of a two-way permutational ANOVA, based on Euclidean distance and 99,999 permutations of residuals under a reduced model. The tested dependent variables were: pre-reproductive time (number of days between introduction of newborn first instar and appearance of first offspring), growth constant k of the exponential growth curve $N = N_0.\exp(kt)$ of the population, and maximum number of aphids attained at a given time. The exponential growth curve was fitted through the aphid counts from day one until the day of the population peak (Fig. 4.1).

A stepwise backward selection procedure was followed for all analyses, only retaining significant predictors ($p < 0.05$). In the case that only one predictor variable was retained in the final model, the test was repeated with unrestricted permutation of raw data, which provides an exact test for the one-way case (Anderson et al. 2008). Pairwise differences between levels of significant factors were tested for each final model by means of a permutational t-test with 99,999 permutations.

Pearson correlations were calculated between the three aphid characteristics and the above mentioned properties of the plants that received aphids. If a significant effect of AMF on an aphid characteristic was found, the above-described ANOVA model was rerun with the plant feature that correlated best with that aphid characteristic included as a covariate.

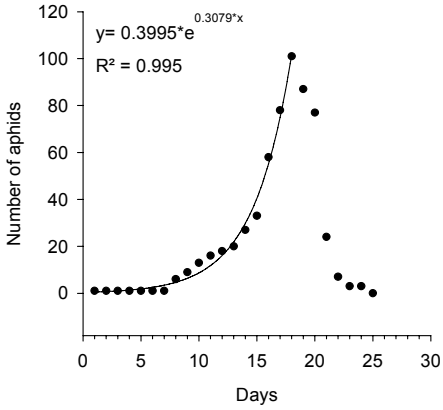


Figure 4.1 An example of the rise and decline of aphid numbers through time. Growth of the population typically follows an exponential curve until a certain peak density is reached, after which the population rapidly crashes. In this case, the pre-reproductive time is 7 days, because at day eight, the aphid reproduced its first own offspring. The equation of the exponential growth curve shows that the growth constant k in this case is 0.3079. The maximum number of aphids here is 101.

4.4 Results

4.4.1 Plants

Aphid herbivory had a dramatic effect on all of the measured plant characteristics (Table 4.1, Fig. 4.2). Aphids caused severe reductions in root and shoot weight and relative water content of the shoot. Although aphids drastically decreased total plant fresh weight, they increased the root fraction of this total weight, indicating that shoot weight was relatively more depressed by aphids than root weight.

There was no main effect of watering treatment on any of the measured plant properties. However, a significant interaction between aphid herbivory and water availability was observed in the effect on root fresh weight (Fig. 4.2a, Table 4.1) The pairwise difference between intermediate and low water levels (pseudo-t = 1.7607, p = 0.0871, 90573 unique permutations) in the

non-aphid group suggests that plants without aphid herbivory developed more roots when the supply of water was lower.

Table 4.1: Results of the three-way permutational ANOVA for the effect of mycorrhizal inoculation, aphid herbivory and watering treatment on plant characteristics. Only results of the final model are displayed for each plant characteristic. Myc: mycorrhizal inoculation treatment (inoculated or non-inoculated). Aphid: aphid herbivory treatment (aphids or no aphids). Water: watering treatment (low, intermediate or high water availability).

Source	df (num., denom.)	Pseudo-F	p	unique permutations
Root fresh weight (g)				
myc	1, 122	23.083	0.00001	90473
water	2, 122	1.36	0.2632	95376
aphid	1, 122	237.97	0.00001	90357
myc x aphid	1, 122	16.684	0.00006	90402
water x aphid	2, 122	3.1233	0.046	95243
Shoot fresh weight (g)				
myc	1, 129	4.2393	0.0402	90350
aphid	1, 129	457.11	0.00001	90535
Shoot dry weight (g)				
myc	1, 127	4.4429	0.0348	90475
aphid	1, 127	349.55	0.00001	90513
Relative water content shoot				
aphid	1, 127	139.47	0.00001	90376
Total fresh weight (g)				
myc	1, 126	11.226	0.0007	90455
aphid	1, 126	550.66	0.00001	90500
myc x aphid	1, 126	7.7057	0.0055	90528
Root fraction of fresh weight				
myc	1, 126	0.010823	0.9178	90427
aphid	1, 126	145.49	0.00001	90348
myc x aphid	1, 126	4.537	0.0347	90395

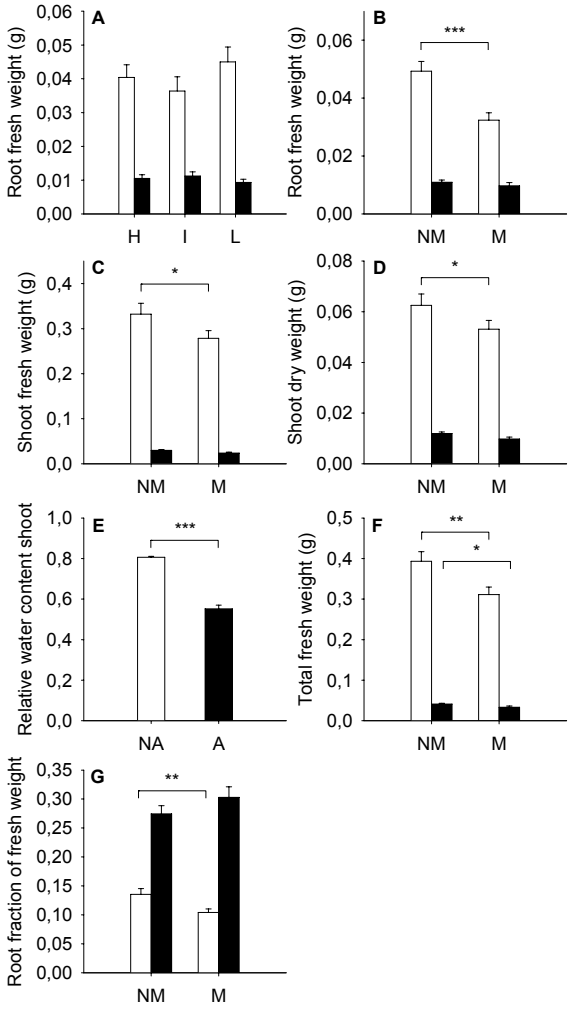


Figure 4.2 Effects of watering treatment, aphid herbivory and mycorrhizal inoculation on different characteristics of *Ammophila arenaria* seedlings (mean ± SE). White bars: no aphid herbivory, black bars: aphid herbivory. L: low water availability, I: intermediate water availability, H: high water availability. M: inoculation with mycorrhizal fungi, NM: no mycorrhizal fungi. A: aphid herbivory, NA: no aphids. Differences between no aphid (white bars) and aphid (black bars) groups within levels of any other treatment were always highly significant ($p < 0.0001$). Differences between levels of treatments within the aphid or non aphid group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Except for the relative water content of the shoots, all plant characteristics were affected by the addition of an AMF spore mixture (Table 4.1, Fig. 4.2). Because of the dramatic reduction of plant biomass by the aphids' feeding, effects of mycorrhizae were more pronounced in the aphid-free plants, leading in some cases to a significant interaction between aphid herbivory and AMF inoculation. Plants that received mycorrhizal inoculum were generally smaller as they displayed a lower root and shoot fresh weight and a lower shoot dry weight. The relative water content of the shoot was unaffected by the AMF treatment (Fig. 4.2e, Table 4.1). So although plants treated with mycorrhizal spores were smaller, their shoot tissues were not drier. The plants that did not receive aphids moreover allocated less of their total biomass to roots when they were inoculated with AMF (Fig. 4.2g, Table 4.1).

4.4.2 Aphids

Neither the growth constant of the exponential growth curve, a measure of population growth rate, nor the time needed by the first instars to develop into reproducing adults were affected by the water availability or mycorrhizal spore inoculation treatment. However, the maximum number of aphids, this is the number that was counted on the peak of the population curve, was significantly lower on plants that had been inoculated with AMF (pseudo-$F_{1,75}$ = 6.2153, p = 0.0153, 839 unique permutations, Fig. 4.3), an effect that was independent of the water availability.

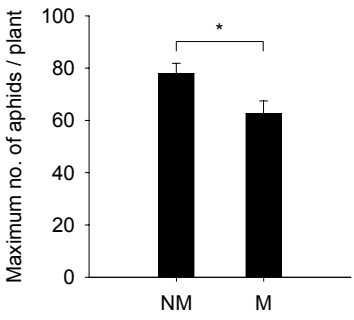


Figure 4.3 Effect of mycorrhizal inoculation of *Ammophila arenaria* seedlings on the maximum number of *Schizaphis rufula* aphids (mean ± SE), i.e. the peak population size. M: inoculation with mycorrhizal fungi, NM: no mycorrhizal fungi. * $p < 0.05$.

The plant characteristic that correlated best with the maximum number of aphids, was the dry weight of the shoot (Pearson's r = 0.80048, p < 0.0001). After correction for the significant effect of shoot dry weight (pseudo-$F_{1,71}$ = 115.96, p = 0.00001, 90441 unique permutations), the effect of AMF on aphid peak number disappeared (pseudo-$F_{1,71}$ = 0.33219, p = 0.5652, 90542 unique permutations).

4.5 Discussion

4.5.1 Plants

There was a negative main effect of AMF on root and shoot biomass of the seedlings. Although AMF are generally considered plant mutualists, several studies have demonstrated that they can cover the spectrum from highly mutualistic to highly parasitic (Hoeksema et al. 2010, Johnson et al. 1997, Klironomos 2003), depending on factors such as species identity of plant and fungus, soil nutrient status and light intensity. However, naturally occurring AMF positively affected biomass of *A. arenaria* plants in a previous study (de la Peña et al. 2006), presumably due to improved plant nutrient uptake. It thus seems unlikely that the species we used in the experiment are inherent parasites of *A. arenaria*. Negative effects of AMF can also arise under circumstances of high availability of the limiting nutrient, when net costs of the mutualism exceed net benefits (Hoeksema et al. 2010, Johnson et al. 1997). The experimental plants were fertilised only once, with a phosphorus-free nutrient solution. The plants were moreover grown on dune sand from their natural environment, known to be poor in plant-available phosphorus (Vandegehuchte et al. 2010c). It thus seems improbable that the association tilted towards antagonism because of the increased nutrient availability. The seedlings were only three weeks old when inoculated with AMF. It is well-known that AMF can hamper seedling growth in the first weeks following germination, when necessary nutrients are obtained from the seed, while carbon is already allocated to the fungus (Johnson et al. 1997). The plants' young age is thus likely to be the most plausible explanation for the observed negative effects of AMF. The observation that relative water content of the shoot was not affected by AMF while

mycorrhizal plants allocated less biomass to roots in the absence of aphids, suggests that AMF actually increased the water extraction efficiency of the seedlings.

The different watering levels did not have substantial effects on plant growth. The only indication of an effect was the observation that *A. arenaria* seedlings without aphid herbivory tended to produce larger root systems when exposed to the lowest water availability. Since no effects of the watering treatment on plant water content were observed, the differential water availability did not cause a stress effect in the plants. In the case of low water availability, the increase in root mass might have alleviated the ultimate water stress. Although mature *A. arenaria* plants are known to thrive in habitats where periods of extreme drought are common, the main cause of death among seedlings is desiccation (Huiskes 1979). Clonal spread is far more important than reproduction by seed in the field, and only in those sites where moisture is more readily available, such as young dune slacks, seedlings can establish (Huiskes 1979). Note that the absence of an effect on plant biomass and water content does not necessarily imply no effect on plant physiology. The increase in root mass of the most stressed seedlings moreover suggests that even small seedlings possess mechanisms to overcome moderately reduced water availability.

In our study, growth of *A. arenaria* seedlings was clearly severely depressed by the aphids, as also observed in previous experiments (Vandegehuchte et al. 2010a, Vandegehuchte et al. 2010c). It seems that the populations of these aphids build up at a very high speed, until the plant is completely crowded and succumbs to the depletion of its nutrients or some other decrease in quality by which the aphids mark the beginning of their own extinction. So under laboratory conditions, these aphid populations seem to be bottom-up controlled by resources. In the field, top-down controlling factors such as parasitoids (M. L. Vandegehuchte, personal observation) and other bottom-up mechanisms such as dispersal induced by crowding probably contribute to the maintenance of low local aphid population densities. Although sometimes appearing in our stock cultures when plants start to wilt, in this experiment no winged morphs were present. The decline of the young seedlings used is very sudden, probably too fast to trigger the development of winged individuals.

4.5.2 Aphids

Aphid populations reached significantly smaller sizes on AMF inoculated plants, while growth rate and pre-reproductive time were unaffected. No effects of watering treatment were found on any of the measured aphid population parameters, thereby confirming that the effect of AMF on aphid population size holds across a range of moisture conditions.

Given the obvious detrimental effect of AMF on the different biomass characteristics of the *A. arenaria* seedlings that did not receive aphids, a sheer reduction of plant size probably caused a concurrent reduction in aphid population size. More importantly, in our study the effect of AMF on aphid population size disappeared when correcting for the strong correlation between shoot dry weight and this aphid population characteristic. Although we did not measure the chemical composition of the plants, this finding strongly suggests that AMF affected aphids merely through changes in plant biomass.

Mycorrhizal fungi can alter both the direct and indirect defence pathways in plants (Bennett et al. 2009, Fontana et al. 2009), which can negatively affect insect herbivores (Kempel et al. 2010, Koricheva et al. 2009). It is also know that mycorrhizal fungi can affect insect herbivores through changes in the nutrient status of the host plant (Gange and Nice 1997, Gange et al. 2005, Goverde et al. 2000). However, if chemical defences or nutrients in the plants would have been the main determinants of aphid population size, our observed result could only have been obtained if plant biomass was highly correlated with chemical defence or nutrient levels. Together with the strong correlation between aphid numbers and shoot dry biomass and the lack of effects on aphid pre-reproductive time and population growth rate, two parameters generally known to be influenced by nutrient status (Khan and Port 2008, Zarghami et al. 2010), this seems very unlikely. Moreover, aphids feed on phloem sap, which is generally poor in defence substances, and a highly specialised aphid species such as *S. rufula* would not be expected to suffer greatly from chemical defences. Therefore, it seems that mechanisms involving chemical plant defence or nutrient status as explanation for the reduced performance of aphids on mycorrhizal plants can be ruled out in our experiment.

Although effects of belowground biota on aboveground biota through regulation of plant primary production have been suggested (Wardle et al. 2004), we are not aware of any study reporting on mycorrhizal fungi – insect herbivore interactions mediated solely by changes in plant biomass. Plant biomass alterations hence present a straightforward but perhaps overlooked mechanism by which mycorrhizal fungi can affect phytophagous insects, of which the generality still needs to be clarified.

5 Contrasting covariation of above- and belowground invertebrate species across plant genotypes*

Martijn L. Vandegehuchte, Eduardo de la Peña and Dries Bonte

Harvest of the *Ammophila arenaria* plants at the end of the field experiment.

* Adapted from: Vandegehuchte M. L., de la Peña E. and Bonte D. in press. Contrasting covariation of above- and belowground invertebrate species across plant genotypes. - Journal of Animal Ecology.

5.1 Abstract

1. Invertebrate species generally do not respond independently to genotypic variation in plants, giving rise to clusters of species that naturally associate with or avoid certain genotypes. This covariation causes coevolution to be diffuse rather than pairwise. Studies on this topic, however, have never considered the belowground invertebrate community, leaving a critical gap in our understanding.

2. We investigated the covariation among naturally colonising above- and belowground invertebrate species across six genetically distinct populations of the dune grass *Ammophila arenaria*. After having grown from seed in a common garden, plants were randomised in a single field site to exclude all but broad-sense genetic variation.

3. Strong positive covariation across genotypes among both above- and belowground invertebrates was detected, while correlations between these two groups were negative. This clustering of above- and belowground species matched well with order level taxonomy. Host range, trophic level and food type on the other hand did not correspond well with the clusters. Within the cluster of aboveground fauna, subsequent groupings were not related to any phylogenetic or ecological characteristic, although correlations within these subgroups were very high. We furthermore demonstrated significant differences in multiple invertebrate species occurrence between plant genotypes, in general as well as at the above- and belowground level.

4. The observed strong covariation suggests diffuse coevolution between *A. arenaria* and its associated invertebrate species. The trade-off between root and shoot invertebrates could however hamper directional selection on resistance to either group.

5. Our results clearly demonstrate the need for studies of plant-animal interactions to include the belowground fauna, as this might drastically alter our general conception of how plants and their associated animal communities interact and how these interactions shape the process of evolution.

5.2 Introduction

It is becoming increasingly clear that invertebrate species do not only distinguish between different host-plant species, but also select on variation in traits within a particular plant species (Johnson et al. 2006). This variation can be a result of the genotype and/or the environment of the plant, or the interaction between both (Falconer and Mackay 1996). If variation in the selected plant traits has a genetic component, plants have the potential to evolve in response to this selection, in turn feeding back to the performance of invertebrates (Toju 2009). Most plant species, however, are host to a plethora of invertebrate herbivores, predators and detritivores (Schoonhoven et al. 2005). A key question in the evolutionary ecology of plant-invertebrate interactions is whether responses of different invertebrates to plant genotypes are correlated or independent (Leimu and Koricheva 2006). If different invertebrate species select on different plant traits, independent responses should occur (Leimu and Koricheva 2006). Negative correlations between invertebrate responses to plant genotypes point at genetically embedded trade-offs between the plant's susceptibilities to different invertebrate species (Leimu and Koricheva 2006). Positive covariation of invertebrate species across plant genotypes, on the other hand, can be expected if invertebrates depend on similar qualitative or quantitative properties of the plant, or when a plant develops generalised defence traits that are effective against a range of invertebrates (Leimu and Koricheva 2006). These different patterns of responses have consequences for the evolution of plant-invertebrate interactions. Genetically uncorrelated responses are a prerequisite to pairwise selection and (co)evolution, while correlated responses imply diffuse selection (Inouye and Stinchcombe 2001, Iwao and Rausher 1997).

Few field studies have investigated the effect of intraspecific genetic variation in plants on the covariation within the associated invertebrate communities (Johnson and Agrawal 2007, Maddox and Root 1990, Newton et al. 2009, Roche and Fritz 1997) In general, these studies provide more evidence of positive covariation of invertebrates across plant genotypes than of negative correlations or independent responses, suggesting a prevalence of diffuse coevolution. Moreover, these studies found little evidence for shared phylogeny or ecological aspects of the covarying invertebrate species.

All these studies focused solely on the aboveground invertebrate community. Most plants, however, harbour a diverse community of belowground invertebrates which rivals its aboveground counterpart in diversity of functions, interactions and degrees of specificity (Boag and Yeates 1998, Brussaard et al. 2007). Although studies on the effect of plant genotypic variation on belowground invertebrates in natural ecosystems are scarce, agricultural systems provide ample evidence of root associated invertebrates selecting on plant genotypes (Anwar and McKenry 2007). The devastation caused by some root-feeding pest species leaves no doubt about the potential impact of belowground invertebrates on plant fitness (Smiley et al. 2005). The 'belowground avoidance, aboveground defence' hypothesis (van der Putten et al. 2001) states that dispersal is a more useful mechanism for plants to escape from belowground antagonists, while the development of direct defences is better suited to withstand attack by mobile aboveground animals. However, this hypothesis assumes aggressive soil pathogens and short-lived plants. Therefore we see no theoretical reason as to why selective pressures imposed by root-associated invertebrates should be less important than those exerted by shoot dwelling invertebrates, especially for perennial plants lacking obvious direct defences. However, compared to aboveground invertebrates, our current understanding of the distinction between plant genotypes by belowground invertebrates is quite limited. Investigating how different above- and belowground invertebrates covary across plant genotypes would provide a first step towards a more complete understanding of the evolutionary ecology of plants and their invertebrate communities. Our aim was to investigate whether root and shoot invertebrates respond similarly, independently or oppositely to plant genotype. The first option would suggest some generalised defence mechanism in the plant to both above- and belowground invertebrates. The second possibility implies selection of root and shoot invertebrates on different and independent plant traits, while the last option would be indicative of some trade-off between susceptibility towards above- and belowground invertebrates.

Two studies have investigated the effect of variation in the dune grass *Ammophila arenaria* (L.) Link on the performance of root-feeding nematodes (de la Peña et al. 2009, Schreck Reis et al. 2008). In both cases, at least some of the tested nematode populations showed different multiplication on

different host plant populations. Genetic differences between the populations of *A. arenaria* that were used in these studies were confirmed by ISSR markers (Rodríguez-Echeverría et al. 2008a). Combined, these studies demonstrate that a broad-sense genetic component of variation between populations affected nematode performance. Given the evidence for effects of plant genotype on the belowground invertebrate fauna and the genetic differentiation between populations of *A. arenaria*, we assessed natural colonisation by above- and belowground invertebrates of different populations of this plant species in a field experiment. We specifically addressed the following questions: 1) does plant genotype affect colonisation by above- and belowground invertebrate species and as such determine the composition of the resulting above- and belowground invertebrate community? 2) to what extent do above- and belowground invertebrate species covary in their responses to plant genotype, and more specifically, are aboveground invertebrates' responses similar to, opposite to or independent from belowground invertebrate responses? 3) is this covariation related to phylogenetical or ecological characteristics of the involved species?

5.3 Material and methods

5.3.1 Study site and system

The experiment was conducted at the Flemish nature reserve 'Westhoek' in De Panne, Belgium (51° 5' N, 2° 34' O). This nature reserve is located at the North Sea coast, and comprises a mixture of dynamic sand dunes, moss dunes, grasslands and shrub thickets. In the dynamic sand dunes, *A. arenaria* is the dominant species, often occurring in large monospecific stands that alternate with open patches of bare sand (Huiskes 1979).

Ammophila arenaria is a native perennial grass, that depends on regular sand burial to maintain vigorous growth (Huiskes 1979). It is distributed along all European coasts south of latitude 63° N. Previous study in the field site has demonstrated that the most abundant invertebrates associated with roots of *A. arenaria* are different root-feeding nematodes belonging to the

genera *Heterodera*, *Pratylenchus* and *Meloidogyne* (Vandegehuchte et al. 2010a). All these genera have been proven to reduce plant performance in at least some cases (Brinkman et al. 2005a, b, de la Peña et al. 2008).

Although less well-studied, the aboveground invertebrate community of *A. arenaria* vegetations is a diverse one, comprising a range of arthropod species, molluscs and woodlice. The community includes herbivores, detritivores and predators, with different herbivores feeding on different plant parts and varying in host range (Huiskes 1979, Weeda et al. 1994).

5.3.2 Setup of field experiment

Seeds of *A. arenaria* were collected each summer from 2002 until 2005 from single populations at six locations in Europe (Fig. S5.3), namely Oostvoorne (the Netherlands: 51° 53' N, 4° 02' O), Het Zwin (Belgium: 51° 21' N, 3° 21' O), De Panne (Belgium: 51° 5' N, 2° 34' O), Westende (Belgium: 51° 9' N, 2° 45' O), Comporta (Portugal: 38° 22' N, 8° 48' W) and São Jacinto (Portugal: 40° 39' N, 8° 44' W). At each location 5 to 10 spikes were collected from 4 plants, standing 25 m apart. The genotype of the populations from Het Zwin, Oostvoorne, São Jacinto and Comporta has previously been characterised by means of ISSR markers (Rodríguez-Echeverría et al. 2008a) revealing genetic differences between all these populations that corresponded to geographical distance. Although the distance between Westende and De Panne is somewhat smaller (17 km) than average distances in the cited study, a study by Hol et al. (2008) demonstrated genetic differentiation between stands of *A. arenaria* in coastal and inland dunes located a mere 200 m apart. Analysis of seedlings of these six populations with AFLP markers confirmed both differentiation between populations and isolation by distance (Vandegehuchte M.L., Breyne P., de la Peña E. & Bonte D., unpublished data).

Seeds were germinated and plants were grown according to a standardised procedure (see Text S5.1). Seventeen months after germination, 10 mature tussocks of each population were transferred to the field. This length of time in a common environment should effectively minimise maternal effects (Johnson et al. 2006). Plants were placed in a randomised design of 6 rows of 10 tussocks at a large open sandy patch in a

dynamic dune area (51° 5' 29.79" N, 2° 34' 8.73" O). This site has been void of any natural vegetation for at least 60 years. The sandy soil did not contain any roots or other fragments and was well homogenised by wind dynamics. Each tussock was carefully removed from its pot, without damaging the roots, and transplanted into the dune soil with the root system freely accessible to soil organisms. Plants were in the field from 22 May until 25 September, 2008.

Plants of each population were clones neither half or full sibs. Additive genetic variation in the plants' associations with different invertebrates could therefore not be estimated. We deliberately chose geographically distant populations in order to maximise the genetic variation between, relative to within, populations. The low variation within populations is probably due to the almost exclusively clonal reproduction of *A. arenaria* in the field (Hol et al. 2008, Huiskes 1979). It therefore seems reasonable to consider each population as a distinct genotype. Moreover, we chose our field site because its uniformity should eliminate environmental differences and interactions with wild stands of *A. arenaria*. Therefore all observed differences between plant populations should be due to broad-sense genetic variation. For ease of reading we used genotype and population as exchangeable terms throughout the text. Our aim was in first instance to determine the variation in the associations of these genotypes with different invertebrate species as such, not the particular plant trait causing this variation. Only the first invertebrate species to arrive is known with certainty to select a plant independently, since subsequent colonisation might be affected by the presence of other invertebrates. These interactions, however, can be considered as part of the natural seasonal assembly process of the community on each plant. The effect of plant genotype on this process hence forms the focus of this paper.

5.3.3 Recording of above- and belowground invertebrate colonisation

All plants were carefully hand searched at eight times throughout the growing season (from 4 July to 24 September, 2008) to assess the occurrence (presence/absence) of aboveground invertebrate species. Occurrence

seemed more appropriate than abundance since the majority of observations of species on plants concerned single individuals, leaving a large proportion of plants unoccupied. Only aphids established large numbers. These colonies, however, were likely founded through parthenogenesis by a single fundatrix that selected a host-plant. We thus decided to record the occurrence of aphid species too, because it allowed objective comparison with the other recorded species. At harvest, all plants were cut off at ground level and the entire root system was dug out. Samples were transferred to the laboratory. Leaves were fresh weighed, oven dried overnight at 65°C and weighed again. The relative water content of the shoots was calculated as the difference between fresh and dry weight divided by the fresh weight. Roots were separated from the bulk soil, of which 500 ml was collected per plant. Roots were washed with tap water, dried with tissue paper, fresh weighed and separated into three equal parts, one of which was stained with acid fuchsin (Baker and Gowen 1996) to visualise root-feeding nematodes in radicula. A second root portion was used to extract nematodes with Baermann funnels, after which the roots were collected from the funnels, macerated and added to the mineral soil. From this combined sample, nematodes were extracted using an automated zonal centrifuge (Hendrickx 1995). For details on the centrifugation protocol, see Vandenbossche (2009). Stained roots were cut into pieces of approximately 1 cm. From each plant, 30 randomly chosen root fragments were inspected under a microscope and nematodes were counted and identified. A fragment that had lost more than half of its cortex was categorised as degenerate, otherwise as vital. A vitality measure for the root system was calculated as the proportion of vital fragments in each sample. Nematodes extracted with modified Baermann technique and zonal centrifugation were identified up to species level, whenever possible. Measurements were prepared from camera lucida line drawings using an Olympus BX 51 DIC microscope. Nematodes visualised in roots were identified and counted as promorphs, which are forms that can be recognised at low magnification. Ten specimens of each promorph were cut out of the root and mounted on glass slides for a more detailed identification, supported by the qualitative data from the Baermann and zonal centrifugation extractions. For analyses (see 5.3.5), we used occurrence data from stained roots and included only nematode taxa that could be identified as such with certainty based on the excised individuals. All

nematode species identifications were based on morphological characteristics.

5.3.4 Selection of invertebrate species for analyses

A diverse array of aboveground invertebrates was observed on the experimental plants over the course of the experiment. The 10 most encountered species were the aphids *Schizaphis rufula* (Walker 1849), *Rhopalosiphum padi* (Linnaeus 1758) and *Laingia psammae* Theobald 1922, the froghopper *Neophilaenus lineatus* (Linnaeus 1758), the leafhopper *Psammotettix maritimus* (Perris 1857), the snails *Theba pisana* (O.F. Muller 1774) and *Candidula intersecta* (Poiret 1801), the woodlouse *Porcellio scaber* Latreille 1804, the harvestman *Phalangium opilio* Linnaeus 1761 and the weevil *Philopedon plagiatum* (Schaller 1783). The majority of the root-feeding nematodes belonged to the species *Meloidogyne duytsi* Karssen et al. 1998, *Heterodera sp.* and *Pratylenchus sp.* (presumably *Heterodera arenaria* Cooper 1955 and *Pratylenchus brzeskii* Karssen et al. 2000). These 13 species were chosen for further analyses. They cover a wide spectrum of taxa, functional groups and degrees of specificity and include herbivores, a predator and a detritivore (Fig. 5.4). Species identifications and ecological characteristics were based on the available literature (Baker and Vogelzang 1988, Bongers 1988, Brzeski 1998, de la Peña et al. 2008, Freude et al. 1981, Gittenberger et al. 1984, Heie 1982, 1986, Holman 2009, Holzinger et al. 2003, Karssen et al. 1998, Karssen and Moens 2006, Nickel 2003, Ribaut 1952, Sternberg 2000, Sutton 1972, van der Stoel and van der Putten 2006, Wijnhoven 2009).

5.3.5 Statistical analyses

Details on the differences in plant characteristics between populations can be found in Text S5.1, Fig. S5.4 and Fig. S5.5.

To investigate whether plant genotype affected the colonisation by aboveground invertebrate species, the effect of plant population on the occurrence of each of the 10 aboveground species was modelled by means of a generalised linear mixed model with a binomial distribution and a logit

link function. Dates before the first or after the last date of presence of each species were excluded to avoid zero inflation. To account for temporal autocorrelation of occurrences on individual plants, the date effect for each plant was modelled as a residual random effect with individual plant as subject. A first-order autoregressive covariance structure was used, as it could be assumed that the correlation between occurrences got higher as dates were situated closer to each other in time. To evaluate the extent to which effects were due to differences in biomass or relative water content, each model was run both with and without these two variables as covariates. We know from previous work that in this system both above- and belowground plant biomass can affect aboveground invertebrates (Vandegehuchte et al. 2010a). Because of the high correlation between root and shoot fresh weight in this case (Pearson's r = 0.78379), only shoot fresh weight was however included as a biomass covariate. Non-significant covariates ($p > 0.05$) were excluded from the model to obtain robust p-values for the other predictors. The Kenward-Roger approximation was used in all cases to calculate the denominator degrees of freedom.

To assess whether plant genotype affected the colonisation by belowground invertebrate species, the effect of plant population on the occurrence of each of the root-feeding nematodes was modelled by means of a generalised linear model with a binomial distribution and a logit link function. Root fresh weight and root vitality are known to potentially affect nematode densities (Vandegehuchte et al. 2010a) and were hence included as covariates in a second model. Non-significant covariates ($p > 0.05$) were excluded from the model.

To investigate to what extent the above- and belowground invertebrate species covaried among plant genotypes, we calculated all 78 pairwise Pearson product-moment correlations between the occurrences of species across the 6 plant genotypes. We used the mean occurrence on each genotype as estimated by the above-described models without covariates. Because it can be expected that 5% of all correlations are significant at the $p = 0.05$ level by chance, a Binomial test was used to assess whether the proportion of significant correlations significantly differed from 0.05 (Zar 1996). To assess whether groups of invertebrates covaried in their occurrence, we used hierarchical cluster analysis based on the species x species matrix of across-genotype correlation coefficients. Prior to analysis,

correlation coefficients were transformed to Euclidean distance by the formula √(1-r). Ward's minimum variance method was used to create a dendrogram that hierarchically groups the 13 invertebrate species, minimising the variance in correlation coefficients within clusters and maximising the variance between clusters. We cut the dendrogram at the level where groups retain 75% of the information compared to individual species in order to obtain robust clusters.

We further investigated whether the covariation captured by the obtained clusters was related to each of five phylogenetical or ecological species traits: taxonomy at the order level, host range (generalist, oligophagous, specialist), food type (leaf tissue, phloem, xylem, prey, detritus, root), trophic group (herbivore, predator, detritivore) and location on the plant (aboveground, belowground). We considered herbivores that fed on *A. arenaria* and maximum four other related grass species as specialists. Species were considered oligophagous if they mainly fed on grasses and to some extent on Juncaceae or Cyperaceae. Generalist herbivores are species that can feed on a multitude of plant families. The detritivore *P. scaber* is a generalist, because it feeds on detritus in almost any habitat type. The predator *P. opilio* is also a generalist, feeding on a wide range of small soft-bodied invertebrates. For each of these five traits we assessed whether the average correlation coefficient among species within groups was greater than the average correlation coefficient among species between groups. The same was done for the statistically derived clusters that served as a control, where we expected the maximum difference between within- and between-group correlations. The significance of differences in correlation coefficient within versus between groups was determined by means of permutational analysis of variance based on Euclidean distance and 99,999 permutations of raw data (Anderson et al. 2008). Because we specifically addressed the question whether above- and belowground invertebrates responded in similar, opposite or independent ways to plant genetic variation, we calculated the average correlation and a one-sided 95% confidence interval (CI) for aboveground invertebrate pairs, belowground invertebrate pairs and pairs composed of an above- and belowground invertebrate.

To assess whether plant genotype affected the overall composition of the above- and belowground invertebrate community, we performed a

permutational multivariate analysis of variance. Since occurrence of the aboveground invertebrate species was measured on several dates, we estimated least squares mean occurrence on each plant using individual plant as a predictor in the above described generalised linear mixed models. We used Bray-Curtis distance and 99,999 permutations of raw data. Analogously, we calculated pairwise differences between genotypes. To assess whether effects of plant genotype were detectable within the above- and belowground invertebrate community, we performed two similar analyses with only the above- or belowground invertebrate occurrences as dependent values.

5.4 Results

5.4.1 Invertebrate responses to genotype

Considering overall differences in aboveground invertebrate species occurrence on plants from different populations, seven out of 10 tested species responded significantly to plant population (Table 5.1). For *L. psammae*, *N. lineatus*, *P. opilio*, *P. scaber*, *P. maritimus* and *R. padi* there was a similar trend of higher occurrence on more local plant populations (Fig. 5.1). Shoot fresh weight proved to be a significant covariate for *P. opilio*, *P. scaber*, *P. maritimus* and *T. pisana* while both shoot fresh weight and shoot relative water content significantly affected the occurrence of *L. psammae* and *N. lineatus*. Inclusion of these covariates only altered the population effect for *T. pisana*, where after correction for biomass differences the population effect disappeared. Note that the pattern for the beetle *P. plagiatum* ($F_{5,174}$ = 2.07, p = 0.07) concurs with the observed trend of higher occurrence on more local plant populations. This species too was not affected by shoot biomass or water content. Two species, *S. rufula* and *C. intersecta*, did not respond to plant population, a result that did not change after correcting for the effect of shoot mass on *C. intersecta* and the effect of shoot mass and water content on *S. rufula* (Table 5.1 and Fig. 5.1). For all species whose occurrence responded significantly to shoot fresh weight, the effect was positive. However, the occurrence of *N. lineatus* and *S. rufula*

increased, while that of *L. psammae* decreased with increased water content of the shoot.

None of the root-feeding nematodes' occurrences was significantly influenced by plant genotype (Fig. 5.2). Inclusion of root biomass and/or root vitality did not significantly improve any of the models.

5.4.2 Covariation of invertebrates across plant genotypes

Correlations between species across plant genotypes varied from -0.73 to 0.97, with an average of 0.31 (SE = 0.055, n = 78). Of all 78 correlations, 14 were significant at the 0.05 level (Fig. 5.3). To obtain such a proportion of significant correlations by chance is highly unlikely (binomial test: $p < 0.0001$). Based on cluster analysis, we defined 4 groups of covarying species (Fig. 5.4). Consistent with our expectations, the mean correlation between species occurrences across plant genotypes within clusters was significantly higher than the mean correlation between clusters (Table 5.2). A very large mean correlation coefficient of 0.81 (SE = 0.034, n = 17) was observed within clusters. The dendrogram displays a first separation between above- and belowground invertebrates. If species are grouped according to their location with respect to the soil surface, this leads to a significant difference between the average correlation of 0.60 (SE = 0.038, n = 48) within groups and the average correlation of -0.16 (SE = 0.070, n = 30) between groups (Table 5.2). The average correlation between aboveground invertebrates was 0.60 (SE = 0.040, n = 45, 95% CI: 0.54 to 0.67), while that between belowground invertebrates was 0.65 (SE = 0.12, n = 3, 95% CI: 0.45 to 0.85), two highly significant results. The average correlation of -0.16 between above- and belowground invertebrate occurrences also turned out to be significant (95% CI: -0.27 to -0.04). This indicates that on average, belowground invertebrates were negatively correlated with the aboveground ones across plant genotypes.

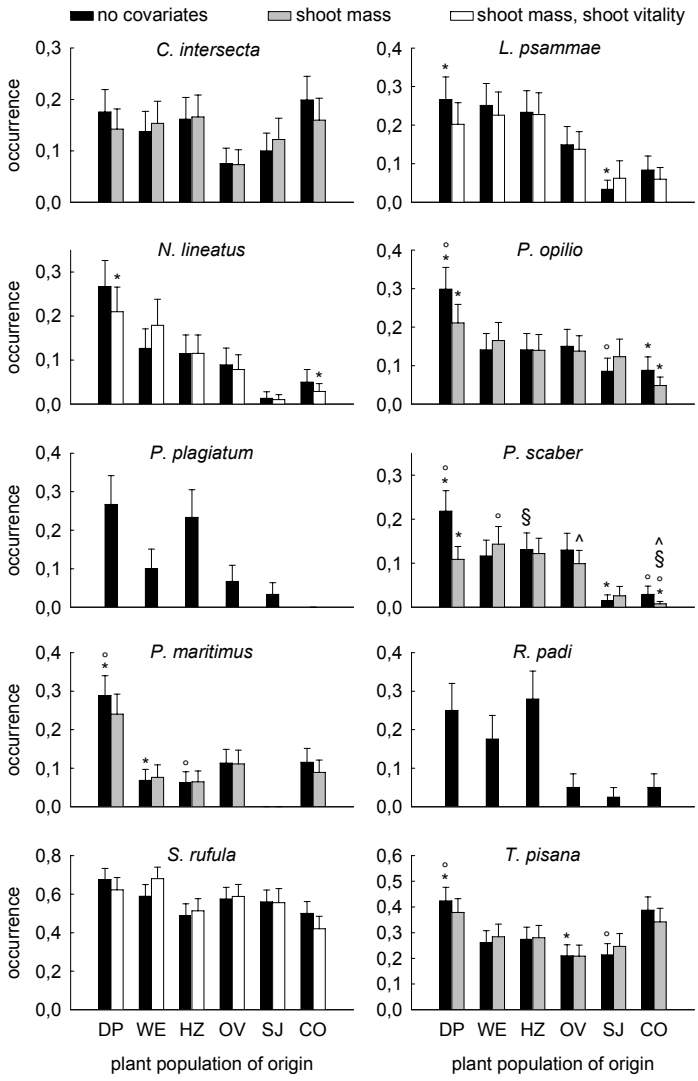


Figure 5.1 Chance of occurrence of the ten most abundant aboveground invertebrate species on *Ammophila arenaria* from different populations. In order of ascending distance to the local population: DP: De Panne – Belgium, WE: Westende – Belgium, HZ: Het Zwin – Belgium, OV: Oostvoorne – the Netherlands, SJ: São Jacinto – Portugal, CO: Comporta – Portugal. Black bars: overall population effect, grey bars: population effect, modelled with shoot fresh weight as a covariate, white bars: population effect modelled with shoot fresh weight and relative water content as covariates (LS means + SE). Significant differences are indicated by same symbols above the bars following Tukey's hsd post hoc test ($p < 0.05$).

Table 5.1 Results of the generalised linear mixed models (F- and p-values) of the effects of *Ammophila arenaria* population of origin on the occurrence of the ten most abundant aboveground invertebrate species, without (left panel) or with (right panel) shoot fresh weight and shoot relative water content as covariates. NS: not significant ($p > 0.05$), and hence excluded from the model.

Source of variation	df	F	p	df	F	p
Candidula intersecta						
population	5, 171.9	1.32	0.2581	5, 171.4	0.77	0.5737
shoot mass				1, 171.4	4.79	0.0300
shoot water content						NS
Laingia psammae						
population	5, 181.4	3.10	0.0104	5, 189.5	2.13	0.0641
shoot mass				1, 189.5	4.33	0.0389
shoot water content				1, 189.5	4.48	0.0357
Neophilaenus lineatus						
population	5, 134.3	3.32	0.0074	5, 136	3.53	0.0050
shoot mass				1, 136	6.09	0.0148
shoot water content				1, 136	3.95	0.0489
Phalangium opilio						
population	5, 149.2	2.90	0.0157	5, 153.8	2.35	0.0436
shoot mass				1, 153.8	14.67	0.0002
shoot water content						NS
Philopedon plagiatum						
population	5, 174	2.07	0.0708	5, 174	2.07	0.0708
shoot mass						NS
shoot water content						NS
Porcellio scaber						
population	5, 181.6	3.42	0.0056	5, 188.3	4.34	0.0009
shoot mass				1, 188.3	33.86	< 0.0001
shoot water content						NS
Psammotettix maritimus						
population	5, 220.7	4.11	0.0014	5, 225.5	2.86	0.0159
shoot mass				1, 176.5	3.74	0.0547
shoot water content						NS
Rhopalosiphum padi						
population	5, 150.3	3.09	0.0110	5, 150.3	3.09	0.0110
shoot mass						NS
shoot water content						NS
Schizaphis rufula						
population	5, 157.4	1.21	0.3061	5, 167.5	1.94	0.0902
shoot mass				1, 167.5	10.85	0.0012
shoot water content				1, 167.5	5.14	0.0246
Theba pisana						
population	5, 174.2	3.38	0.0061	5, 174.6	1.41	0.2241
shoot mass				1, 174.6	5.81	0.0170
shoot water content						NS

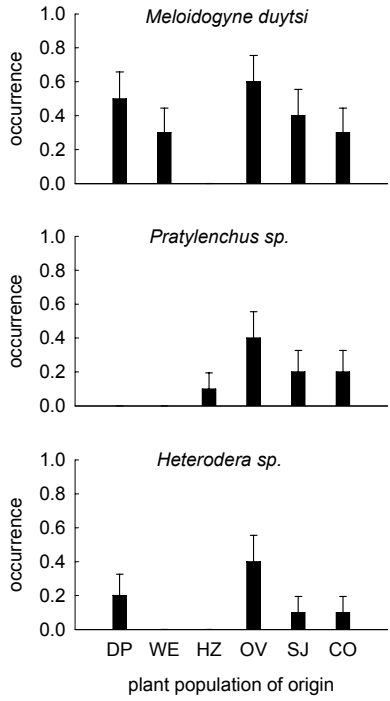


Figure 5.2 Chance of occurrence of the three most abundant root-feeding nematodes on *Ammophila arenaria* from different populations. In order of ascending distance to the local population: DP: De Panne – Belgium, WE: Westende – Belgium, HZ: Het Zwin – Belgium, OV: Oostvoorne – the Netherlands, SJ: São Jacinto – Portugal, CO: Comporta – Portugal. Bars represent LS means + SE. There are no significant differences.

Mean correlations within the different trophic groups were actually significantly lower than between groups (Table 5.2), implying that trophic level did not contribute to the observed clustering. The mean correlation between invertebrates with the same food type was not significantly different from the correlation between food types (Table 5.2), although a trend of higher within group correlations was observed. The host range of species poorly matched the clusters (Table 5.2), while correlations were significantly higher within than between order level taxa (Table 5.2). In summary, the hierarchical clustering first split off the belowground invertebrates (Fig. 5.4, cluster D), all root-feeding Tylenchida, and within the remaining group of aboveground invertebrates then separated a group of two leaf-feeding Pulmonata (cluster A). The remaining species, all arthropods, were grouped in two clusters (B and C), which did not correspond well with any of the phylogenetic or ecological groupings.

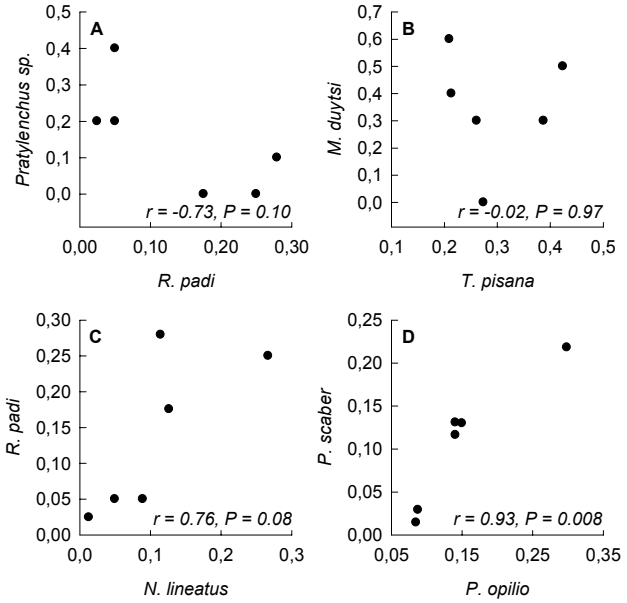


Figure 5.3 Examples of pairwise Pearson product-moment correlations between the occurrences of invertebrate species across the six genotypes of *Ammophila arenaria*. These examples demonstrate the range of observed correlations.

5.4.3 Effect of plant genotype on community composition

The effect of plant genotype on the composition of the 13-species invertebrate community was highly significant (pseudo-$F_{5,54}$ = 3.07, p = 0.00001). Pairwise differences between plant populations in their invertebrate community composition were significant at the $p < 0.05$ level in 10 out of 15 cases. The 10-species aboveground invertebrate community was also significantly affected by plant genotype (pseudo-$F_{5,54}$ = 3.48, p = 0.00001) with 10 out of 15 pairwise differences between plant genotypes being significant. Although the effect of plant genotype was not significant for any of the individual nematode species, the effect on the group of nematodes was significant (pseudo-$F_{5,54}$ = 2.71, p = 0.0124). The nematode community differed significantly for 3 out of 15 pairs of plant genotypes. We visualised the relationship between plant genotype and the invertebrate community by means of a Nonmetric Multidimensional Scaling ordination (see Text S5.2 and Fig. S5.6).

	location	trophic group	food type	host range	taxon	cluster	
	A	H	leaf	G	Pul	A	candinte
	A	H	leaf	G	Pul	A	thebpisa
	A	H	phloem	S	Hem	B	lainpsam
	A	H	leaf	O	Col	B	philplag
	A	H	phloem	G	Hem	B	rhoppadi
	A	H	xylem	O	Hem	C	neopline
	A	P	prey	G	Opi	C	phalopil
	A	D	detritus	G	Iso	C	porcscab
	A	H	phloem	S	Hem	C	psammari
	A	H	phloem	S	Hem	C	schirufu
	B	H	root	S	Tyl	D	meloduyt
	B	H	root	O	Tyl	D	hetespec
	B	H	root	S	Tyl	D	pratspec
$r_g W$	0.60***	0.22	0.55	0.34	0.66**	0.81***	
$r_g B$	-0.16	0.52*	0.27	0.3	0.24	0.17	

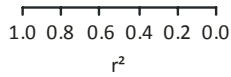


Figure 5.4 Dendrogram depicting the covariation of the 13 most abundant invertebrate species across the six genotypes of *Ammophila arenaria*, based on hierarchical cluster analysis. We considered clusters at the 75% information level. Species are furthermore grouped by order level taxonomy (Pulmonata: Pul, Hemiptera: Hem, Coleoptera: Col, Opiliones: Opi, Isopoda: Iso, Tylenchida: Tyl), host range (generalist: G, oligophagous: O, specialist: S), food type (leaf tissue, phloem, xylem, prey, detritus, root) and trophic level (herbivore: H, predator: P, detritivore: D). We compared the average across-genotype correlation of species occurrences within the clusters/groups (r_g W) with the average correlation of species occurrences between the clusters/groups (r_g B). Significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Species names on the dendrogram are a combination of the first four letters of the genus followed by the first four letters of the species epithet or spec if the species was uncertain.

Table 5.2 Difference in correlation coefficient across six plant genotypes of pairs of invertebrate species within- versus between groups. Invertebrate species were grouped according to ecological characteristics as well as based on hierarchical cluster analysis. Significance of differences was tested by means of permutational analysis of variance based on Euclidean distance and 99,999 permutations of raw data.

Grouping variable	df	pseudo-F	p
location (above/below)	1, 76	107.69	0.00001
trophic group	1, 76	6.62	0.012
food type	1, 76	3.7	0.058
host range	1, 76	0.088	0.77
taxon	1, 76	9.52	0.0029
statistical cluster	1, 76	31.14	0.00002

5.5 Discussion

5.5.1 Response of invertebrate species to plant genotype

Our results demonstrate that genetic variation in *A. arenaria* can affect the colonisation by invertebrate species. At the individual species level, aboveground invertebrates tended to differentiate more strongly between plant genotypes than belowground invertebrates. For only one of the aboveground species the effect could be attributed to biomass differences, while differences in relative water content did not affect the genotype effect (Table 5.1). This implies that most of the aboveground invertebrates selected on differences of a more subtle physiological nature, such as in nutritional quality or leaf toughness. Although the occurrence of each of the nematode species did not significantly differ between plant genotypes, their combined occurrence did. This can be explained by the positive covariation between the three nematode species and the enhanced statistical power of the multivariate analysis. Considering the large number of aboveground species that responded significantly to plant genotype, it was not surprising that the effect of plant genotype was highly significant on the aboveground community of invertebrate species, as well as on the entire community. This is the first study to demonstrate that intraspecific genetic variation in plants has the potential to structure both the above- and belowground invertebrate community.

Differential colonisation of naturally occurring plant genotypes by aboveground arthropod species is widely documented (Fritz and Price 1988, Fritz 1990, Orians and Fritz 1996, Pilson 2000, Shen and Bach 1997, Stinchcombe and Rausher 2001, Tikkanen et al. 2003). One study by Crutsinger et al. (2008) demonstrated that the foliage-based arthropod diversity and abundance on *Solidago altissima* L. clones were strongly affected by plant genotypic identity, while the litter-based community only showed weak responses. Interestingly, the species showing the strongest response to *A. arenaria* genotype was *P. scaber*, a detritivore. Therefore, more plant species should be investigated before generalisations can be made about the responses of litter feeders to intraspecific genetic variation in plants.

A large body of literature documents on the genetic base of differences between cultivars of crop species in their resistance to root-associated invertebrates, nematodes in particular (Fuller et al. 2008). Similar results for non-crop plant species are however limited. In contrast to the laboratory multiplication experiments by Schreck Reis et al. (2008) and de la Peña et al. (2009), we could not detect species level responses of nematodes to plant genotypic identity. However, since we considered occurrence instead of abundance, and because nematodes in the field rarely reach laboratory densities, the detection level of responses in our study could have been considerably lower. This conjecture is further confirmed by the fact that taken together, the community of three nematode species showed significant differences in occurrence between genotypes.

5.5.2 Covariation of invertebrate species across plant genotypes

Cluster analysis based on a matrix of across-genotype correlations between invertebrate species' occurrences revealed a large degree of covariation between species across plant genotypes. Interestingly, the first division in the dendrogram was that between above- and belowground invertebrates, indicating that, as groups, they selected differently on plant genotype. Not surprisingly, of all tested ecological and phylogenetic groupings of invertebrate species, the location with respect to the soil surface matched the clusters best. Invertebrate species belonging to the same order also clustered together, and there was a tendency for species within clusters to forage on the same food type. Our conclusions therefore would have been drastically different if we had only considered the aboveground component of the invertebrate community. If we remove the cluster of nematodes (D) from the dendrogram (Fig. 5.4), the average correlations within and between groups only differ for the statistically derived clusters (A, B and C). This implies that the matching with order level taxonomy and food type was due to the first separation of a cluster of root-feeding nematodes of the order of Tylenchida.

In a meta-analysis on genetic correlations between plant resistances to different aboveground enemies, Leimu and Koricheva (2006) found that

across 29 studies, the average correlation was 0.1470 and ranged between - 0.833 and + 1. From this they concluded that positive covariation between enemies of plants is likely common, suggesting a prevalence of generalised defences against enemies. We found positive covariation at the higher end of this spectrum within both the above- and belowground invertebrate community. However, taking into account the large amount of negative correlations between above- and belowground invertebrates lowers the average correlation to 0.31 and broadens the range to levels more comparable with those found by Leimu and Koricheva (2006). It therefore seems that responses to genetic variation in *A. arenaria* are strongly correlated within both the above- and belowground invertebrate fauna while aboveground invertebrates respond to this variation oppositely to their belowground counterparts.

Leimu and Koricheva (2006) furthermore found no relationship between genetic correlations and the feeding guild of herbivores, but found an overall positive genetic correlation for pairs of specialists or generalists, while specialists were on average not correlated with generalists. This conclusion is nuanced by Johnson and Agrawal (2007) who conclude that in the case of arthropods, host specificity is likely to be a weak predictor of covariation across plant genotypes (Maddox and Root 1990, Roche and Fritz 1997). Indeed, also in our study, host range is not explicative of the observed covariation in invertebrate responses, whether or not the belowground fauna is included.

5.5.3 Implications

The observed large amount of significant correlations between invertebrate species across plant genotypes suggests that selection imposed by these species on *A. arenaria* is diffuse rather than pairwise. Moreover, the positive covariation within the above- and belowground component of the invertebrate fauna suggests selection for generalised resistance traits both at the root and shoot level. However, the observed trade-off between above- and belowground invertebrate occurrence can be an ecological cost constraining the directional evolution of either type of resistance. This can cause stabilising selection to preserve intermediate levels of resistance to both groups of invertebrates and explains why some studies fail to detect the

costs associated with resistance (Strauss et al. 2002). The cost of increased aboveground resistance might be an increased vulnerability to root herbivores, rather than some reduced allocation of resources to other plant functions or resistance to other aboveground enemies.

We did not experimentally exclude the different invertebrate species in a factorial design. Our measure of across genotype correlation thus could be based on ecological interactions where one invertebrate species affects the occurrence of another. For example, herbivores might avoid plants already attacked by other herbivores (Leimu and Koricheva 2006). Since the more mobile shoot herbivores arrived first on the plants, they might even have affected subsequent colonisation by root feeders in this system (Vandegehuchte et al. 2010a), causing the observed trade-off. Indeed, there is a large body of literature documenting on possible interactions between the above- and belowground fauna (van der Putten et al. 2001, Vandegehuchte et al. 2010a, Wardle et al. 2004). We furthermore did not assess fitness effects of the different invertebrates. However, each of the observed nematode species has proven to be able to reduce plant biomass. The most common aboveground species, the aphid *S. rufula*, has proven detrimental for the plant under laboratory conditions (Vandegehuchte et al. 2010a). The assumption that at least some of the investigated species have the potential to affect plant fitness therefore seems reasonable. Moreover, whether correlated responses of invertebrates are caused by truly genetic traits, such as linkage disequilibrium or pleiotropy, or by some invertebrate species selecting on certain genotypes and subsequently altering the effect on plant fitness by other species, both imply that potential coevolution will be diffuse (Inouye and Stinchcombe 2001, Iwao and Rausher 1997, Stinchcombe and Rausher 2001). If the aim is to gain understanding in the natural selection on variation in plants by invertebrates, associations with these invertebrates therefore have to be measured under natural circumstances, including the natural interactions between species (Roche and Fritz 1997). It furthermore seems practically unfeasible to selectively exclude each of 13 different above- and belowground species.

Our conclusions hold consequences for the study of coevolution in general. The issue of pairwise versus diffuse coevolution has until now only been investigated aboveground. Our results, however, indicate that the selection imposed by invertebrates associated with plant roots could very

well be operating in the opposite direction of selection by aboveground invertebrates. Research on other plant-invertebrate systems could elucidate the generality of our results and add to the understanding of the role of belowground invertebrates in the coevolution between plants and their invertebrate communities.

From a conservational point of view, our results stress the necessity to choose *A. arenaria* populations for sand fixation carefully (van der Putten 1990). As *A. arenaria* is a keystone species, changes in the genetic variation underlying its associated biotic interactions might have ecosystem consequences (Whitham et al. 2003). A more holistic approach of the study of ecological communities, including the belowground component, could thus greatly enhance our understanding of the mechanisms and trade-offs in plant defence and the process of coevolution and provide more effective tools for species conservation.

6 Non-local genotypes of a resident plant species reduce invertebrate species richness and alter food web structure*

Martijn L. Vandegehuchte, Eduardo de la Peña, Peter Breyne and Dries Bonte

A dune area in the Westhoek nature reserve, characterised by a high level of sand dynamics, with vigorous *Ammophila arenaria* stands.

---

* Adapted from: Vandegehuchte M. L., de la Peña E., Breyne P. and Bonte D. under review. Non-local genotypes of a resident plant species reduce invertebrate species richness and alter food web structure. - Ecology.

6.1 Abstract

Effects of the genotypic identity of a plant can extend beyond the individual phenotype to the community and ecosystem. Because man is translocating plant material at an increasing rate, introductions of alien plant genotypes that are difficult to distinguish from native ones are probably common. Even though such introductions can cause cryptic invasions, their effects on local communities remain largely unexplored.

Ammophila arenaria is used throughout the world for dune stabilisation and has become invasive in several regions. We used this grass as a model system to address the impacts of the introduction of non-local genotypes on the diversity and food web characteristics of the local invertebrate community. We installed a common garden in the field, consisting of a mixture of the local population and five introduced populations obtained from regions throughout the natural range, and identified all naturally colonising invertebrates. Different measures of species diversity and food web composition were assessed.

Although total and herbivore alpha diversity as well as food web link density, generality and vulnerability decreased on introduced genotypes in relation to geographical distance to the field site, predator and detritivore diversity matched this ordering less well. Food web connectance tended to increase on introduced populations, indicating that the observed decline in the other food web metrics was caused by an overall decrease in food web size. Species turnover was not related to genetic distance between populations.

Our study demonstrates that introduction of non-local genotypes of a resident plant species can be detrimental to the associated invertebrate community. On the one hand this confirms the idea that caution should be exerted when selecting plant material for restoration or sand stabilisation purposes. On the other hand, the observed release from herbivores of introduced genotypes of *A. arenaria* raises questions about the role of aboveground enemy release in the invasiveness of this species in other continents, which until now has only been addressed in the context of belowground antagonists.

6.2 Introduction

A large body of literature provides evidence of the effects of plant species diversity on the diversity of the associated community of arthropods (Siemann et al. 1998, Knops et al. 1999, Haddad et al. 2009). More recently, the role of intraspecific genotypic diversity in structuring the diversity of animal communities has received increasing attention (Crutsinger et al. 2006, Johnson et al. 2006). Research has demonstrated that genetic variation in plant populations can affect ecological functioning at different scales, from the individual plant phenotype to the associated ecological community and further to the entire ecosystem (Crutsinger et al. 2006, Bailey et al. 2009, Madritch et al. 2009). This finding has given rise to the concept of the genetic assembly rule (Bangert and Whitham 2007) and the redefinition of the extended phenotype (Dawkins 1982) in the context of community and ecosystem genetics (Whitham et al. 2003, Whitham et al. 2006). Different studies have assessed the effect of plant genetic identity by comparing the associated fauna of different populations or genotypes, often using hybrids (Dungey et al. 2000, Hochwender and Fritz 2004, Bangert et al. 2005, Hochwender et al. 2005, Wimp et al. 2005, Tovar-Sanchez and Oyama 2006, Wimp et al. 2007, Johnson 2008, Mooney and Agrawal 2008, Barbour et al. 2009a, Barbour et al. 2009b, Crutsinger et al. 2009, Schadler et al. 2010). The majority of these studies conclude that different plant genotypes harbour different invertebrate communities, often associated with quantitative differences in species richness.

Over the past few decades, the literature on the consequences of invasive plant species has boomed (Richardson and Pysek 2008). A less recognised, but potentially very common type of biological invasion is the so-called cryptic invasion, in which invaders can not, or at least not easily, be recognised from native taxa. Cryptic invaders can be alien genotypes of a native species (Saltonstall 2002), or even alien genes that become established in native populations through hybridisation (Petit 2004).

One of the best documented cases of cryptic invasion is that by an introduced genotype of common reed (*Phragmites australis*) into North America (Saltonstall 2002). The European M haplotype has managed to spread throughout the North American continent, and has already replaced

the local *P. australis* subsp. *americanus* in certain areas. Park and Blossey (2008) have shown that the invasive genotype had a longer life span and optimised leaf-age structure. The M haplotype was furthermore less attacked by *Lipara* gallflies and *Hyalopterus pruni* aphids and gallfly attack led to stunted growth. This study thus suggests that both greater resource capture and enemy release might contribute to the success of introduced *P. australis*.

Ammophila arenaria (L.) Link is a perennial grass native to all coastal dunes of Europe south of latitude 63° N. This species needs regular burial by wind-blown sand to maintain vigorous growth, and starts to decline as soon as sand-drift ceases, giving other plant species the opportunity to invade the vegetation, thereby marking the next stage in the natural succession series of dunes (Huiskes 1979). Because of its ability to stabilise sand dunes, *A. arenaria* has been used for erosion control for a very long time in Europe (Green 1965). Because of the success of *A. arenaria* as a sand binder, the species has been introduced in regions across the world, including Australia, New Zealand, South Africa, Palestine, India, the Falkland Islands and the west coast of the USA (Green 1965, van der Putten et al. 2005). In Australia (Heyligers 1985), New Zealand (Hilton et al. 2005) and the USA (Wiedemann and Pickart 1996), *A. arenaria* is now considered invasive. In South Africa, its spread seems limited by water availability (Peter 2000), although the persistence of the planted populations and the potential for invasion have raised concern and the question for more research (Hertling and Lubke 2000).

The decline of *A. arenaria* in stabilised dunes has been attributed to soil-borne pathogens in its native range (van der Putten et al. 1988, van der Putten et al. 1993), presumably root-feeding nematodes (Brinkman et al. 2005b, van der Stoel et al. 2002) or pathogenic fungi (De Rooij - Van der Goes 1995). Therefore, the release of soil-borne enemies has been hypothesised as the principal cause of the species' invasiveness. Experimental testing so far yielded no, or at best conditional, evidence for a role of root-feeding nematodes or pathogenic fungi in the invasiveness of this grass (Beckstead and Parker 2003, Knevel et al. 2004, van der Putten et al. 2005), while changes in aboveground arthropod communities have been attributed to the presence of introduced *A. arenaria* (Slobodchikoff and Doyen 1977, Webb et al. 2000).

Although the species is not invasive in Europe, cryptic introductions of non-native genotypes into local populations are likely, because *A. arenaria* plants are still used on a large scale for sand stabilisation purposes. For example, in the dunes of Voorne, 120 ha of foredune ridge have been fortified with *A. arenaria* (van der Putten and Kloosterman 1991). If non-native genotypes are used for such massive plantings, the consequences could be analogous to those of an invasion. Cryptic invasion has already been demonstrated for the North American *A. breviligulata*, where some genotypes used for dune restoration and stabilisation differ from the local native genotypes (Fant et al. 2008). Given the invasiveness of *A. arenaria* and its reported effects on native invertebrate communities in other continents, such cryptic introductions might not be without consequence. In a previous study, we could demonstrate that 7 out of 10 common aboveground invertebrate species were less frequently encountered on introduced plants from more distant populations. If anything, root-feeding nematodes preferentially colonised the alien populations (Vandegehuchte et al. 2010b).

In this paper, we investigate the invertebrate communities on the local plant population and five European populations from locations of widely differing geographic distance to the field site. We assess whether the species diversity of the entire aboveground invertebrate community differs between plant genotypes, and whether these differences are more pronounced for herbivores, predators or detritivores. In addition to diversity measures, we calculate some statistics developed in food web ecology to investigate differences in invertebrate community structure among plant populations, which are hard to detect with traditional survey techniques (Memmott 2009). A source food web approach is appropriate, given the fact that *A. arenaria* mainly occurs in monospecific stands but harbours a diverse invertebrate community (Memmott et al. 2000). We furthermore test whether the effective species turnover between populations, expressed in multiples of mean species richness of a population, depends on the geographical and/or genetic distance between those populations.

We hypothesise that not only introduction of *A. arenaria* into ecosystems where it is absent, but also introduction of *A. arenaria* populations into ecosystems where a native *A. arenaria* population is present, can affect both the diversity and food web structure of the local invertebrate community. We hypothesise that populations from distant locations exert larger effects

on the local diversity and that species turnover increases with distance between populations. We specifically address the question whether introduced populations support less herbivore species, and whether these herbivores are more vulnerable to predation, since these observations would point at the possibility of enemy release.

6.3 Material and methods

6.3.1 Setup of field experiment

The field experiment has been described in chapter 5. It was conducted in the dunes of the Flemish nature reserve 'Westhoek' in De Panne, at the Belgian coast. In this reserve, large areas of drifting sand can still be found. In these dynamic sand dunes, *A. arenaria* is the dominant plant species, often occurring as monospecific stands.

From 2002 until 2005, seeds of *A. arenaria* were collected each summer from single populations at six locations in Europe, namely Oostvoorne (the Netherlands), Het Zwin (Belgium), Westende (Belgium), De Panne (Belgium), São Jacinto (Portugal) and Comporta (Portugal). At each location 5 to 10 spikes were collected from 4 plants, standing 25 m apart.

Seeds were germinated and plants were grown according to a standardised procedure. Seventeen months after germination, 10 mature tussocks of each population were transplanted to a large sandy patch in the dunes (51° 5' 29.79" N, 2° 34' 8.73" O), with the roots free in the soil. Plants were randomised in a setup of 6 rows of 10 tussocks. They were left in the field from 22 May until 25 September 2008. Details of the growing procedure and field setup can be found in chapter 5.

Maternal effects should be effectively minimised by the length of time the plants spent in a common environment (Johnson et al. 2006). Moreover, the homogeneity of our field site should eliminate environmental differences between individual plants as well as interactions with wild stands of *A.*

arenaria. Therefore all observed differences between plant populations should be due to broad-sense genetic variation.

6.3.2 AFLP analyses

The genetic variation of the six *A. arenaria* populations was investigated by means of the Amplification Fragment Length Polymorphism (AFLP) technique. Details of the used protocol can be found in Text S6.1.

6.3.3 Recording of invertebrates

At eight occasions between 4 July and 24 September 2008, all plants were carefully hand searched to assess the presence of aboveground invertebrate species. At harvest all plants were cut off at ground level and transferred to the laboratory. Leaves were oven dried overnight at 65°C and weighed. Adult invertebrates of the orders Araneae, Coleoptera, Collembola, Dictyoptera, Hemiptera, Isopoda, Lepidoptera, Opiliones, Pulmonata and of the family Formicidae of the order Hymenoptera were identified to the species level using published identification keys based on morphological characteristics. Adults of the orders Psocoptera, Dermaptera, Diptera, Acari and Hymenoptera (excluding Formicidae) as well as all larval or nymphal stages were classified into morphospecies, based on a personal assessment of their external similarity. In total, 79 species and 21 morphospecies were recorded. Of those 21 morphospecies, 14 were represented by a single individual. The large majority of invertebrates could thus be identified to the species level.

6.3.4 Invertebrate diversity measures and food web characteristics

The alpha diversity of each plant was determined as the number of species recorded on that plant at least once. Whittaker's effective species turnover ($\beta_{Mt\ -1}$) was used as a measure of beta diversity (Tuomisto 2010a). This is the total or gamma diversity divided by the average alpha diversity, subtracted by one to set the minimum value to zero. We followed a pairwise

approach (Tuomisto 2010b) by calculating pairwise effective species turnover values between compositional units. This was done both at the plant and population level. Since each population was represented by ten replicate plants, five independent pairwise combinations of plants could be made per population. Between the six populations, 45 pairwise species turnover values could be calculated.

Each diversity measure was calculated for a full dataset of all encountered species and a reduced dataset only retaining species with a known ecological association with *A. arenaria*. This way potential noise in the data caused by the presence of vagrant or transient invertebrate species could be ruled out. We subsequently repeated the calculations for herbivores, for predators and omnivores, and for detritivores from the dataset of associated species, in order to investigate whether diversity patterns differed according to trophic function.

Food web statistics were calculated by excluding rarely caught species (species occurring less than 4 times on a plant tussock). This resulted in a reduced dataset comprising 5 detritivores, 3 omnivores, 16 herbivores and 13 generalist predators (see Fig. S6.3). Predator-prey links were derived by applying a combined size-species based food web approach. Generalist predators feed on herbivores, detritivores, omnivores and predators, but prey suitability is determined by relative differences in size and thus depends on the specific life stage reached by predators during the summer season (Cohen et al. 1993, Nentwig 1987, Wise 1995). Previously documented life cycle reconstructions based on temporal changes in body size (Bonte and Maelfait 1998, Bonte et al. 2000, Bonte and Maelfait 2001) were used to calculate differences in size between prey and predators during the summer season. In consequence, we do not use data on size at adulthood but instead size during the specific season when potential prey are present to reconstruct prey-predator links. A link is only considered when (a) species show an overlap in niche and (b) when relative differences in size are in the range of 50-80% at the moment when both predator and prey are present (Nentwig 1987). Web-building spiders that are able to catch prey reaching sizes of 150% their own body size were not recorded. When prey and predator show distinct niche separation in *A. arenaria* tussocks, for instance due to different day/night activities (Bonte et al. 2006), or the use of different strata in the tussock (soil versus plant tissue, detritus versus leaves;

Bonte and Vandegehuchte, unpub. data) they were not considered realistic to form a link. Cannibalism in spiders depends predominantly on size differences (Vanden Borre et al. 2006) and was therefore only considered in the species with a biennial life cycle (*Tibellus maritimus*), that subsequently has two distinct size cohorts in which the 2-year old individuals are approximately double the size of individuals in their first year. The other species were annual, attaining roughly the same size at the same moment during the season. Because of low densities and small size differences, cannibalistic events are not expected (Wise 1995). For omnivores, the same rules were used to determine links with prey. Besides the previously recorded species richness of herbivores, detritivores and predators, four additional food web statistics (see e.g., Memmott et al. 2000, Pimm et al. 1991) were calculated based on the number of species or food web size (S) and realised links (L) per tussock: linkage density (L/S), connectance (L/S^2), prey vulnerability (the number of predators linked to a prey), averaged across the different prey species, and predator generality (the number of prey linked to a predator), averaged across the different predatory species.

6.3.5 Statistical analysis

To assess the degree of molecular variation within and among populations, total genetic diversity was partitioned by applying a hierarchical analysis of molecular variance (AMOVA) on Euclidean pairwise genetic distances using GENALEX 6.1 (Peakall and Smouse 2006). Significances were determined based on 999 permutations. The Φ_{PT} is an analogue for F_{ST}-values used for dominant markers such as AFLP, and was derived from the Euclidean genetic distances. Its significance was calculated using the Monte Carlo procedure in GENALEX 6.1 (999 permutations). Unbiased expected heterozygosity was also calculated using GENALEX 6.1, following the approach of Lynch and Milligan (1994).

The effects of plant population, shoot dry mass and their interaction on the alpha diversity were tested with a permutational ANOVA based on 99,999 permutations of residuals under a reduced model, using type III sums of squares. Dry mass of the shoot was included as a covariate in the analysis of alpha diversity, because of the expected species-area relationship. Shoot dry mass was significantly positively correlated with the number of tillers at

planting ($p = 0.0062$) and at harvest ($p < 0.0001$), the length of leaves at planting ($p = 0.0001$) and at harvest ($p < 0.0001$), and the root fresh weight at harvest ($p < 0.0001$). It is therefore considered a good proxy for the overall plant size during the course of the experiment. The effect of plant population on the pairwise beta diversity, the connectance, the link density, the average herbivore vulnerability and the average predator generality were tested with a permutational ANOVA based on 99,999 unrestricted permutations of raw data, which provides an exact test for the one-way case. Pairwise differences between treatment levels were tested by means of a permutational t-test based on 99,999 permutations.

In all analyses, homogeneity of variances was tested with a permutational Levene's test based on 99,999 permutations. Note that for permutational ANOVA, there are no explicit assumptions regarding the distribution of the original variable. Variances were equal (Levene's test, $p > 0.05$) for all variables, except for food web connectance and prey vulnerability. For these two variables, the variance-weighted one-way ANOVA of Welch (1951) was performed, after food web connectance data were raised to the power ¾ in order to normalise residuals.

We investigated directional trends in each of the tested variables, by means of an ordered heterogeneity (OH) test (Rice and Gaines 1994a, b). We ranked the six plant populations in order of geographic distance to the local population. Subsequently, the Spearman's rank correlation (r_s) between these distance ranks and the ranks of the means of the tested variable was calculated. The complement of the p-value of the population effect in each of the above described ANOVAs (p_c) was then multiplied with the obtained Spearman's rank correlation. This measure $r_s p_c$ increases when the heterogeneity among populations increases in a fashion that is consistent with the ordering prescribed on the alternative hypothesis. Because we did not have any a priori hypothesis about the direction of this ordering, the alternative hypothesis stated that the order of variable means, be it ascending or descending, was monotonically related to the order of the populations. Therefore a two-tailed p-value for the $r_s p_c$ statistic was obtained from Rice and Gaines (1994a).

A Mantel test was performed to investigate whether the genetic distance between populations was correlated with the geographical distance. We

furthermore used Mantel tests to assess whether the pairwise effective species turnover between populations was related to either the geographical or genetic distance between those populations. All Mantel tests were based on 99,999 permutations.

6.4 Results

6.4.1 AFLP analyses

Based on the 46 polymorphic AFLP markers scored, the unbiased expected heterozygosity of populations ranged from 0.046 (SE = 0.019) to 0.338 (SE = 0.026), with an average of 0.122 (SE = 0.011) across all plants and populations. The AMOVA indicated that 74% of the total genetic variance could be attributed to the difference between populations, leaving 26% of the variance to be found within populations. This Φ_{PT} of 0.74 had a p-value of 0.001. Pairwise population Φ_{PT} values were significant for all pairs of populations, with the exception of De Panne and Oostvoorne, where the Φ_{PT} was 0.057, a difference that was found to be statistically insignificant (p = 0.125, see Table S6.2).

The correlation between the geographic distance and Nei's genetic distance between plant populations was 0.9954, a significant result according to the Mantel test (p = 0.02425). This means that genetic isolation by distance is present for the populations we tested.

6.4.2 Invertebrate diversity

The alpha diversity of invertebrates associated with a plant, based on all species, differed significantly according to an interaction between shoot dry mass and plant population (Table 6.1). As expected, the number of species increased with larger shoot biomass, but the strength of this increase differed between plant populations (Fig. 6.1a). For example, even on plants from Comporta with a large biomass, the number of species remained relatively low. The main effect of plant population was highly significant,

even after correction for the effect of shoot biomass. The OH test revealed a significant consistency of the effect with the order of geographical distance from De Panne (Table 6.2). Plants from Belgian populations supported a larger diversity of invertebrates compared to plants from the Netherlands and Portugal (Fig. 6.1a). Very similar results were obtained when the analysis was restricted to invertebrate species with a known ecological association with *A. arenaria* (Table 6.1, Table 6.2, Fig. 6.1b). The observed pattern was thus little influenced by the inclusion of transient species from other habitats or species with an unknown host plant affiliation.

Table 6.1 Results of the ANOVAs testing the effect of shoot dry mass and plant population on the alpha diversity of different groups of species. Mass: shoot dry mass, pop: plant population of origin.

Source	df	SS	MS	Pseudo-F	p	unique perms
alpha diversity all species						
mass	1,48	410.33	410.33	66.674	0.00001	90476
pop	5,48	162.33	32.467	5.2756	0.0006	95263
mass x pop	5,48	173.72	34.744	5.6456	0.0004	95245
alpha diversity associated species						
mass	1,48	305.08	305.08	69.195	0.00001	90475
pop	5,48	147.02	29.405	6.6692	0.00008	95269
mass x pop	5,48	128.09	25.618	5.8103	0.0003	95173
alpha diversity associated herbivores						
mass	1,48	23.258	23.258	13.5	0.0006	90386
pop	5,48	49.384	9.8767	5.733	0.0003	95197
mass x pop	5,48	10.771	2.1541	1.2504	0.303	95210
alpha diversity associated predators and omnivores						
mass	1,48	90.525	90.525	59.954	0.00001	90555
pop	5,48	27.727	5.5455	3.6727	0.0077	95234
mass x pop	5,48	52.008	10.402	6.8889	0.00005	95217
alpha diversity associated detritivores						
mass	1,48	9.7934	9.7934	15.383	0.0002	90492
pop	5,48	0.4371	0.08742	0.13731	0.983	95260
mass x pop	5,48	7.3129	1.4626	2.2973	0.056	95267

The effect of shoot dry mass on the alpha diversity of herbivores associated with *A. arenaria* was significant, and after correcting for this relationship, the effect of plant population was still significant (Table 6.1). The effect of shoot biomass on herbivore diversity did not significantly differ between plant populations. Again, the number of herbivore species per

population decreased in order of their distance to the field site, so higher numbers were found on Belgian plant populations (Table 6.2, Fig. 6.2a).

The alpha diversity of predatory and omnivorous invertebrate species, known to occur in *A. arenaria* stands, increased significantly with shoot dry biomass. The strength of this relationship significantly differed between plant populations (Table 6.1), but the main effect of plant population was highly significant. In the case of predators and omnivores, the order of the mean numbers of species did not match the order of the geographic distances of the populations to the local population (Table 6.2). This can be seen in Fig. 6.2b, where it is clear that for an average shoot weight, the number of species is highest on plants from São Jacinto, but lowest on plants from Comporta.

Table 6.2 Results of the ordered heterogeneity test for the different diversity and food web metrics. r_s: Spearman's rank correlation between the order of plant populations according to their distance to the local population and the means of the tested variable per population. Means were corrected for possible covariates in the model. $p_c = 1 - p_F$. p: two-tailed probability value. Note that for the food web connectance and the prey vulnerability, the F-statistic is obtained from Welch's variance-weighted ANOVA. For the other variables the F-statistic is from a permutational ANOVA based on 99,999 permutations.

Source	df	F	p_F	r_s	$r_s p_c$	p
α total	5,48	5.2756	0.0006	-0.8857	-0.8852	0.001
α associated	5,48	6.6692	0.00008	-0.8857	-0.8856	0.001
α herbivore	5,48	5.733	0.0003	-0.7714	-0.7712	0.006
α predator	5,48	3.6727	0.0077	-0.4286	-0.4253	0.14
α detritivore	5,48	0.13731	0.983	-1	-0.017	0.86
β total	5,24	0.56663	0.7238	0.71429	0.19729	0.37
β associated	5,24	0.60881	0.6957	0.88571	0.26952	0.26
β herbivore	5,24	1.8838	0.1356	0.31429	0.27167	0.26
β predator	5,24	1.85	0.1414	0.65714	0.56422	0.049
β detritivore	5,24	0.94564	0.4731	0.46382	0.24439	0.36
density	5,54	5.3086	0.0005	-0.8857	-0.8853	0.001
connectance	5,23.8	1.41	0.2562	0.71429	0.53129	0.061
vulnerability	5,24.6	16.75	0.0001	-0.7714	-0.7714	0.006
generality	5,43	8.8239	0.00002	-0.8286	-0.8286	0.0032

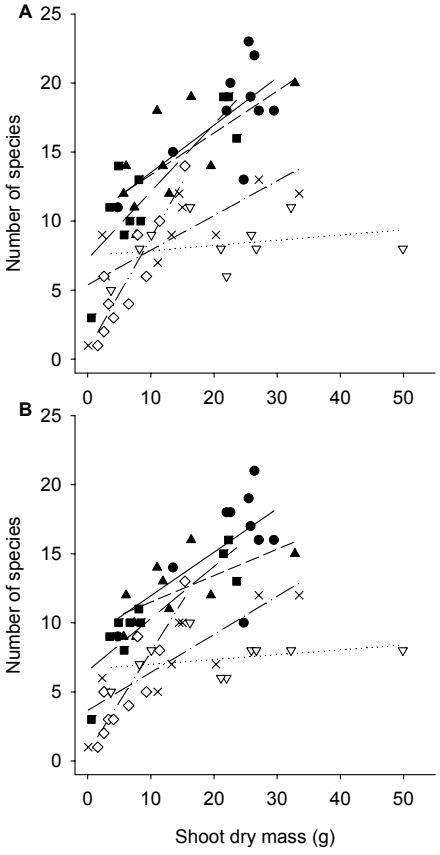


Figure 6.1 Relationship between dry shoot mass of a tussock and the number of invertebrate species encountered in that tussock, for six different populations of *A. arenaria*. A) All invertebrate species included. B) Dataset restricted to invertebrate species with a known ecological association with *A. arenaria*. B: Belgium, N: the Netherlands, P: Portugal.

● ——— : De Panne (B)
■ — — — : Westende (B)
▲ - - - - - : Het Zwin (B)
× — · — · — : Oostvoorne (N)
◇ — · · — · · : São Jacinto (P)
▽ ····· : Comporta (P)

The number of detritivore species that are known to occur in *A. arenaria* vegetations, was significantly affected by the dry mass of the shoot. No significant main effect of plant population was detected, but the difference between plant populations in the strength of the effect of shoot mass on detritivore diversity was marginally significant (Table 6.1). The steepest increase in detritivore species number with shoot dry mass was observed for the Belgian populations, while this increase was weaker for plants from São Jacinto and Oostvoorne. On plants from Comporta, the number of detritivore species even slightly decreased with increasing shoot biomass (Fig. 6.2c). The order of the corrected mean numbers of detritivore species matched the order of the populations' distance to the field site perfectly (r_s = -1). Although the increased power of the OH test lowered the p-value in comparison to

that of the ANOVA, the effect of population still remained highly insignificant.

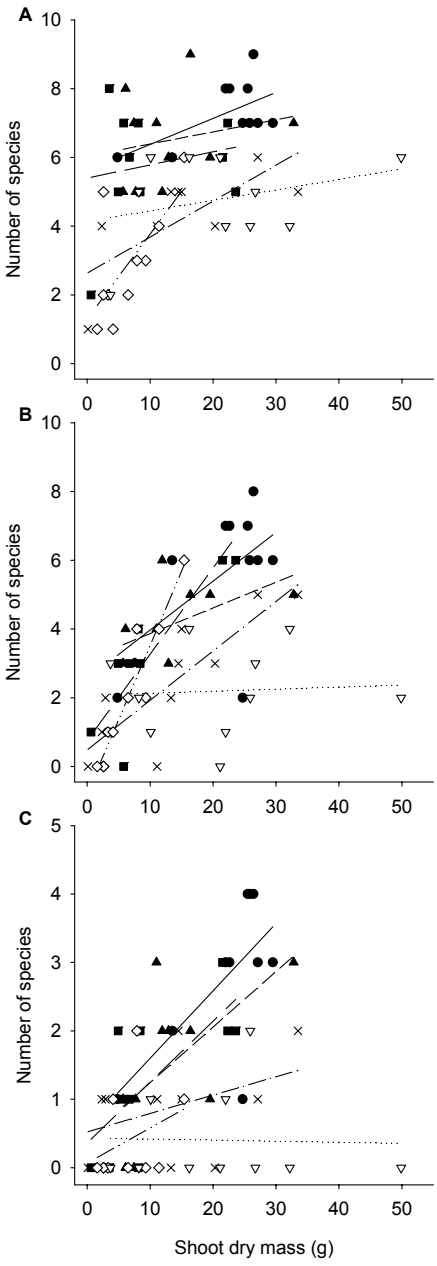


Figure 6.2 Relationship between dry shoot mass of a tussock and the number of invertebrate species encountered in that tussock, for six different populations of *A. arenaria*. Only species with a known ecological association with *A. arenaria* are included. A) Herbivores. B) Predators and omnivores. C) Detritivores. B: Belgium, N: the Netherlands, P: Portugal.

● ——— : De Panne (B)
■ – – – – : Westende (B)
▲ - - - - - - : Het Zwin (B)
× —·—·— : Oostvoorne (N)
◇ —··—·· : São Jacinto (P)
▽ ····· : Comporta (P)

For each of the five tested species groupings, Mantel tests revealed that Whittaker's effective species turnover was not significantly correlated with either the genetic or the geographic distance between plant populations. This implies that the difference in species identity between plant populations, whether it concerned all invertebrate species, associated species, herbivores, detritivores or predators and omnivores, was similar between genetically and geographically more or less related plant populations.

The effect of plant population on the pairwise Whittaker's effective species turnover was not significant for any of the five tested species groupings ($p > 0.05$), when tested by means of ANOVA. However, the OH test revealed a significant difference between plant populations in the average turnover of predators and omnivores, in the a priori expected order of those populations based on their distance to the field site (Table 6.2, Fig. 6.3). Indeed, apart from the Oostvoorne population, the pairwise species turnover increases with geographical distance. This means that the difference in species composition between two randomly chosen plants was similar for all plant populations, when all invertebrates, only herbivores, or detritivores were considered.

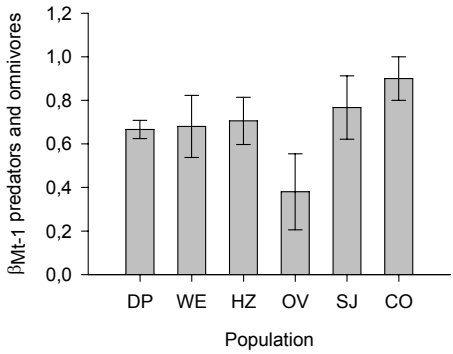


Figure 6.3 Effect of plant population on Whittaker's effective species turnover (mean ± SE) between two *A. arenaria* tussocks from a same population. Populations are represented in order of ascending geographical distance from the local population. DP: De Panne (Belgium), WE: Westende (Belgium), HZ: Het Zwin (Belgium), OV: Oostvoorne (the Netherlands), SJ: São Jacinto (Portugal), CO: Comporta (Portugal).

6.4.3 Invertebrate food web characteristics

The link density differed significantly between plant populations (pseudo-$F_{5,54}$ = 5.3086, p = 0.0005). Link density tended to be higher for plant populations from the Belgian coast, leading to a significant OH test (Table 6.2, Fig. 6.4a). Welch's variance-weighted ANOVA revealed that the effect of plant population on the power-transformed food web connectance was not statistically significant ($F_{5,23.8}$ = 1.41, p = 0.2562). However, because the highest connectance was found in food webs on plants from São Jacinto, followed by those on Comporta plants, the increased power of the OH test resulted in a borderline significant effect of population (Table 6.2, Fig. 6.4b).

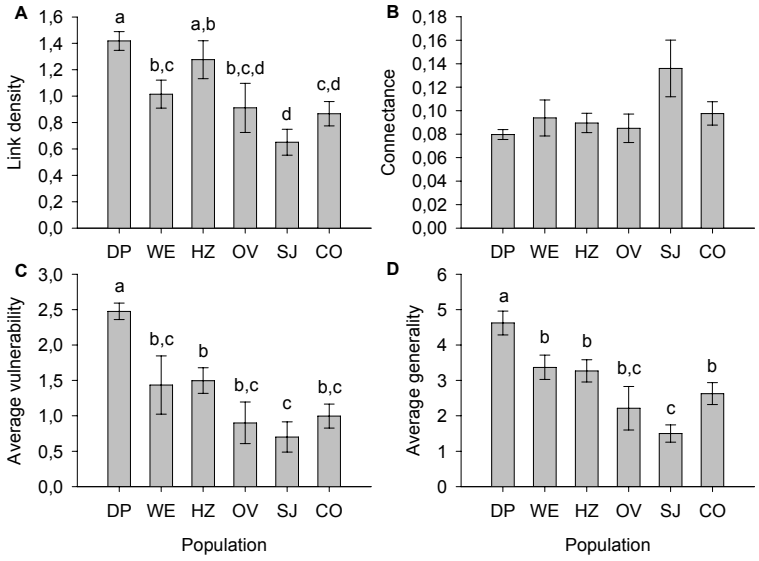


Figure 6.4 Effect of plant population on characteristics (mean ± SE) of the body size based food web associated with an *A. arenaria* tussock. A) Link density. B) Connectance. C) Average vulnerability of a prey, i. e. the number of predatory species that consume a prey species. D) Average generality of predator, i. e. the number of prey species that a predatory species consumes. Populations are represented in order of ascending geographical distance from the local population. DP: De Panne (Belgium), WE: Westende (Belgium), HZ: Het Zwin (Belgium), OV: Oostvoorne (the Netherlands), SJ: São Jacinto (Portugal), CO: Comporta (Portugal). Significant pairwise differences are indicated by different letters above the bars (p < 0.05).

Plant population significantly affected the average prey vulnerability ($F_{5,24.6} = 16.75$, $p < 0.0001$). Prey species were on average more vulnerable to predation on the local plant population, i.e. the population from De Panne, compared to all other plant populations (Fig. 6.4c). Plants from Het Zwin supported prey species with a higher vulnerability than those on plants from São Jacinto. Not surprisingly, the OH test also yielded a highly significant p-value (Table 6.2). The average generality of predators significantly differed between plant populations (pseudo-$F_{5,43} = 8.8239$, $p = 0.00002$). Note that the pattern of the predator generality closely resembles that of the prey vulnerability (Fig. 6.4d), with again a significant OH test of the effect of plant population (Table 6.2). Predators found in tussocks from De Panne are on average more general than those found in stands from any other population, while the predator generality is lowest for plants from São Jacinto.

6.5 Discussion

Introduction of non-local plant populations clearly affected the alpha diversity of the invertebrates in a negative way. This effect was most pronounced for herbivorous species whose species richness decreased with increasing genetic and/or geographic distance of the introduced genotype. Single species' responses (see Vandegehuchte et al. 2010b) thus scale up to the level of the alpha diversity of the entire community.

The average vulnerability of prey species, in our case almost exclusively herbivores, was significantly lower for all introduced plant populations. A strikingly similar pattern was observed for the average generality of predators. These results are consistent with the observed decreased link density of food webs on introduced plant populations. Indeed, if the number of species, or nodes, on each trophic level decreases linearly, the number of possible links decreases exponentially, and therefore the number of possible links per species decreases. The connectance, i.e. the number of realised links relative to the total number of possible links, tended to increase with increasing genetic distance to the native genotype. Therefore, the observed pattern was not caused by a decrease in the proportion of interactions relative to the available species. Thus, food webs on introduced plant populations had less links both from predators to prey and vice versa,

because of the depauperate herbivore and predator species richness, but not because of a lower proportion of realised links.

Based on the selectively neutral AFLP-markers, the Mantel test indicated significant isolation by distance of the used plant populations. This was mainly due to fact that both the genetic and geographic differences were relatively large between the Portuguese and all other populations as well as among the Portuguese populations. If the latter are excluded, the correlation between genetic and geographic distance disappears ($r = -0.16$, $p = 0.5$). All populations were significantly differentiated based on their AFLP-profiles, except for De Panne and Oostvoorne, although of all North Sea populations, these two populations were located furthest apart. Interestingly, the Oostvoorne population and the Portuguese populations showed comparable differences to the Flemish populations in the alpha diversity of the different tested groups of invertebrates. Also the link density, average vulnerability and average generality differed significantly between the North Sea populations, with the largest differences occurring between the local population from De Panne and the Oostvoorne population. Differences between the North Sea populations in their invertebrate community diversity therefore did not correspond well with their genetic differences as revealed by selectively neutral markers. From previous work, we know that several invertebrate species occurred more frequently on the local plants than on plants from Oostvoorne (Vandegehuchte et al. 2010b). Thus, also at the species level, the invertebrate responses were not in line with the neutral genetic differences between plant populations of the North Sea coast. This suggests that genetic differences in quantitative traits under selection rather than neutral shifts in genetic structure are responsible for some of the divergent invertebrate community patterns. Such is also the case for the species richness of the bark-dwelling arthropod community of Eucalypt trees in Southern Australia, which is tightly linked to genetic variation for the type and quantity of decorticating bark, but does not correlate well with selectively neutral genetic variation (Barbour et al. 2009). Indeed, quantitative traits are likely to be subject to selection, leading to both phenotypic divergence of parapatric races and phenotypic convergence of allopatric races, as shown for Eucalypt trees by Steane et al. (2006). Quantitative traits of *A. arenaria* prone to selection could relate to desiccation resistance, such as root morphology and efficiency, leaf hairs and

longitudinal grooves, as well as traits related to defence, such as leaf toughness, silica content or secondary metabolites.

Our expectation that species turnover would increase with genetic distance between the populations was not confirmed. This indicates that different plant populations harboured some different species, but that these differences were similar between all populations. As in the study of Eucalypt arthropods (Barbour et al. 2009), the genetic similarity rule (Bangert et al. 2006) which states that plants that are more genetically similar (at a molecular level) support more similar communities, is therefore not supported by our results.

At the moment, the success or failure of particular plant invaders remains essentially unpredictable. One of the prevalent hypotheses in this context is the 'Enemy Release Hypothesis' (ERH), which states that introduced plants have the potential to spread rapidly because they are liberated from their coevolved natural enemies (Keane and Crawley 2002, Maron and Vila 2001). Rigorous testing of the ERH has however begun only very recently. In a meta-analysis Liu and Stiling (2006) found that plants have higher numbers of phytophagous insects in their native than in introduced ranges, with a higher percentage of specialist and endophagous insect species, which they considered support for the ERH. However, Chun et al. (2010) found that although most invasive plant species incurred less damage than natives, this did not always result in greater performance, indicating that enemy release could explain the success of invaders in only some cases. The only studies investigating the impact of herbivores on native and introduced plants through herbivore exclusion experiments both showed support for the ERH (DeWalt et al. 2004, Schierenbeck et al. 1994). However, all these studies considered invasive plant species. To our knowledge, the only study to date investigating the success of an invasive plant genotype in the context of natural enemies, is that by Park and Blossey (2008) on *Phragmites australis*. Our study demonstrates that introduced populations of *A. arenaria* supported less herbivorous invertebrate species than the local population. Even populations from short geographical distances, that are genetically not very differentiated, such as in our case the Oostvoorne population, can reduce the diversity of the invertebrate community, and in particular that of invertebrate herbivores, substantially. The decreased average vulnerability of these herbivores on introduced populations, as

explained above, is a mathematical consequence of the overall decrease in both predator and herbivore species richness. Our previous study moreover indicated that most of the abundant invertebrate species occurred significantly less on at least some of the introduced populations, sometimes even when these originated from locations very nearby (Vandegehuchte et al. 2010b).

The probability that cryptic introductions of *A. arenaria* are actually occurring is high. Although this has not been studied directly in Europe it is a common practice to introduce culms of *A. arenaria* for dune stabilisation. In these events, large extensions of dune are planted, presumably not always with plants of the local genotype. Furthermore, it has been demonstrated in North America that some of the populations of *A. breviligulata* used for restoration purposes genetically differ from the native populations (Fant et al. 2008). Moreover, our observation that De Panne and Oostvoorne plants are genetically similar, based on neutral markers, while the genetically different populations from Westende and Het Zwin are geographically located in between them, suggests a historical translocation event between De Panne and Oostvoorne.

The implications of these results are twofold. On the one hand, they stress the importance of choosing local *A. arenaria* populations to obtain plant material for the use in dune stabilisation, as cryptic introduction of other populations, even at geographically small scales, can have negative impacts on the associated invertebrate community. We thereby confirm the hypothesis of Vander Mijnsbrugge et al. (2010) that introduced genotypes can negatively affect local herbivore species, which they consider one reason for choosing local plant material for ecological restoration, apart from better known phenomena such as maladaptation of non-local populations or outbreeding depression caused by hybridisation.

On the other hand, although we did not quantify the effect of herbivore damage on the performance of the different plant populations, the observation that herbivore loads were reduced on introduced populations raises the question whether release of aboveground enemies might play a role in the invasiveness of *A. arenaria* in other continents. So far, the enemy release hypothesis has only been addressed for the belowground antagonistic fauna of *A. arenaria*, (Beckstead and Parker 2003, Knevel et al.

2004, van der Putten et al. 2005), but convincing evidence for the generality of the ERH in the context of soil biota has not been put forward yet. Given the considerable diversity of aboveground herbivores that forage on *A. arenaria*, it is surprising that these have never been considered as possible actors in the ecology of this grass, both in the native and introduced ranges. Although our study did not investigate *A. arenaria* in the regions where it is invasive, it demonstrates that introducing genotypes from outside of the local population can lead to significantly lowered aboveground herbivore species richness. Exclusion experiments in the native and introduced ranges should therefore elucidate the importance of aboveground antagonists in the invasiveness of *A. arenaria*.

Our study shows that cryptic introductions of *A. arenaria* genotypes can have far-reaching negative consequences for the local invertebrate fauna associated with this grass, even at small geographical scales. Caution should therefore be exerted when choosing populations for use in restoration or stabilisation projects. Our experiment furthermore serves as a model system that can provide insight into the effects of introductions at a larger taxonomic and geographic scale, for example the introduction of the European *A. arenaria* into areas of the USA where the local *A. breviligulata* naturally occurs. If the same mechanisms apply to genotypes and species, *A. arenaria* might be experiencing release from aboveground herbivores in the introduced ranges, which could be part of the explanation of the species' invasiveness. Furthermore, given the ever-increasing translocation of organisms around the globe, introductions of non-local genotypes into native communities are expected to increase in frequency. The detection of such cryptic introductions and the investigation of the mechanisms by which they affect communities and ecosystems thus pose new challenges for future ecologists. The integration of theoretical and practical knowledge from the largely independent fields of community genetics and invasion ecology might generate a useful tool to address these challenges.

7 General discussion

7.1 Overview of the main conclusions

In the previous chapters, we have explored some of the interactions between above- and belowground organisms associated with *Ammophila arenaria* in the laboratory as well as in the field. We have decoupled the effects of the abiotic and biotic soil component on the performance of this grass and an aboveground herbivore in a laboratory experiment. In addition, we have assessed the natural colonisation by invertebrates of different populations of *A. arenaria* in a common garden in the field, to determine the covariation of above- and belowground invertebrate species across genotypes and to investigate the impact of cryptic introductions of non-local genotypes on different diversity measures and the food web structure of the aboveground invertebrate community. This research has led to the following main conclusions:

- Under controlled environmental conditions, a natural community of root-feeding nematodes negatively affected aboveground-feeding aphids of the species *Schizaphis rufula*, and vice versa, the presence of these shoot-feeding aphids negatively impacted the belowground nematodes. No negative correlation between nematode and aphid numbers could, however, be detected in the field. Instead, variation in nematode numbers could be explained by the vitality and density of the roots, while aphid abundance correlated best with plant height, root density and leaf vitality. The hypothesis that interactions under controlled conditions can be mitigated by other environmental actors in a field situation was thus confirmed.

- In the constant environment of the laboratory, seedlings of *A. arenaria* were more affected by the biotic than by the abiotic soil component. They grew larger on sterile soils than on soils inoculated with biota from either dynamic or stabilised dunes, although this effect was observed only for one of three dune regions where soil was obtained. Plant biomass was larger on sterile soil from this region compared to sterile soils from the other regions, indicating that also abiotic differences between dune regions affected plant growth. The observation that the root fraction of the seedlings' dry weight was increased by inoculation with soil biota from dynamic dunes was

detectable across the three dune areas where soil was sampled. *S. rufula* aphids bred on these seedlings were only affected by the region of origin of the soil. Since this effect was irrespective of whether soils were sterile or inoculated with biota, it had to be caused by abiotic differences between the dune areas. Although several abiotic soil characteristics of the different treatment soils were quantified, none of them could consistently explain the performance of these aphids. Hence, the hypothesis that plants perform better on sterile soils from dynamic than from stabilised dunes could not be confirmed by this study. However, as predicted, plant growth was depressed by inoculation with soil biota. Since aphid performance was only affected by abiotic differences between dune areas, the hypothesis that aphids would perform better on sterile soils and on soils from dynamic dunes could not be confirmed. As hypothesised, both plants and aphids were affected by the soil's area of origin, which emphasises the importance of spatial replication.

- In a similar laboratory setup, the inoculation with AMF negatively affected several performance parameters of *A. arenaria* seedlings. Population sizes of *S. rufula* aphids were negatively affected by AMF inoculation, although individual aphid development time as well as the growth constant of the exponential growth curve were unaffected. When including shoot dry biomass of the seedlings as a covariate, the effect of AMF on aphid population sizes moreover disappeared. These findings strongly suggest that a sheer reduction in plant biomass, and thus available primary resources, led to the negative effect of AMF on aphid populations. These results are in contrast with the hypothesis that plants and aphids would benefit from the presence of AMF. The young age of the used seedlings might explain this discrepancy.

- When exposed to natural colonisation by above- and belowground invertebrates in a common garden in the field, different genotypes of *A. arenaria* affected the occurrence of different species of invertebrates. Strong positive covariation across genotypes in the occurrence of different aboveground invertebrate species could be detected. Similarly strong positive covariation was found in the responses of different root-feeding nematode species to plant genotype. However, correlations between the above- and

belowground invertebrate species were negative on average. The hypothesis of positive covariation of aboveground invertebrates was thus confirmed. The negative covariation between root and shoot invertebrates suggests a trade-off between the plant's susceptibility to either group of organisms.

- Several of the introduced plant genotypes in the field experiment supported a significantly lower species richness of aboveground invertebrates, a pattern that was most pronounced for the herbivorous species. The depauperate herbivore richness on introduced plant populations, together with the low predator richness on some of these non-local plant genotypes, led to a decrease in predator generality, prey vulnerability and link density of the food web. As hypothesised, introduction of non-local plant genotypes affected both the diversity and food web structure of the local invertebrate community. However, the prediction that species turnover increases with distance between plant populations could not be confirmed. Genetic isolation by distance, based on neutral markers, was mainly caused by the presence of two Portuguese plant populations. These populations also supported a strongly decreased invertebrate diversity. However, invertebrate responses were not in line with the neutral genetic differences between plant populations of the North Sea coast. This suggests that genetic differences in quantitative traits under selection rather than neutral shifts in genetic structure caused some of the invertebrate community differences.

7.2 Implications for conservation

Several of the above-stated conclusions have implications for conservation. First of all, it is important to notice that the dynamic dune areas with high rates of sand accretion on which the vigour of *A. arenaria* depends, are becoming rare. Throughout northwest Europe, a tendency of increasing dune fixation and vegetation succession over the past century has been observed. In a review on this topic, Provoost et al. (2010) list several reasons for this. These include changes in land use, such as the decrease in livestock grazing and the increase in fixation of dunes for coastal protection,

an overall decline in storminess, tall grass encroachment induced by atmospheric N deposition and the drastic reduction of rabbit numbers by myxomatosis. Furthermore, it is not unlikely that the effects of global change could exacerbate the increase in phytomass and acceleration of succession. At a more local scale, human infrastructure development has caused fragmentation of the dune landscape, thereby disrupting geomorphological processes. Moreover, the presence of economically valuable assets in the proximity of dunes is a further incentive to fixate the dunes. Another side effect of urbanisation is the introduction of non-native plant species that have the potential to escape and some of which have already become invasive in the dunes. In conclusion, most of the recent changes in vegetation cover of coastal dunes are due to anthropogenic influences. Areas dominated by *A. arenaria* showed a steep decline over the past century, being mainly replaced by scrub or tall grassland, and are now almost restricted to foredunes. This trend has been observed in Belgium and elsewhere in Europe. Furthermore, the amount of energy needed for the wind to destabilise dunes is much greater than the decrease in energy needed to stabilise dunes, making destabilisation of former stabilised dunes a very difficult task (Tsoar 2005).

Half of the Belgian coastal dune area is currently urbanised. In the remaining dunes, scrub and woodland increased at the expense of open dune habitats, mostly due to plantation. The contribution of spontaneous succession to woodland is limited (Provoost et al. 2010). Aerial photographs of the area around the Westhoek nature reserve (Fig. 7.1) revealed a spectacular decrease in mobile dune, comprising bare sand and *A. arenaria* vegetation, of 60% between 1948 and 2005 (Provoost and Van Landuyt 2001). From aerial photographs taken during World War I and landscape descriptions and photographs (Massart 1912), we know that the dune landscape was almost free of woody vegetation at that time. More than 100 scrub and woodland species present in the dunes today were not found before 1940 (Provoost and Van Landuyt 2001). Furthermore, the proportion of non-native plant species along the Belgian coast increased from 5% to 20% over the last 50 years (Provoost et al. 2010).

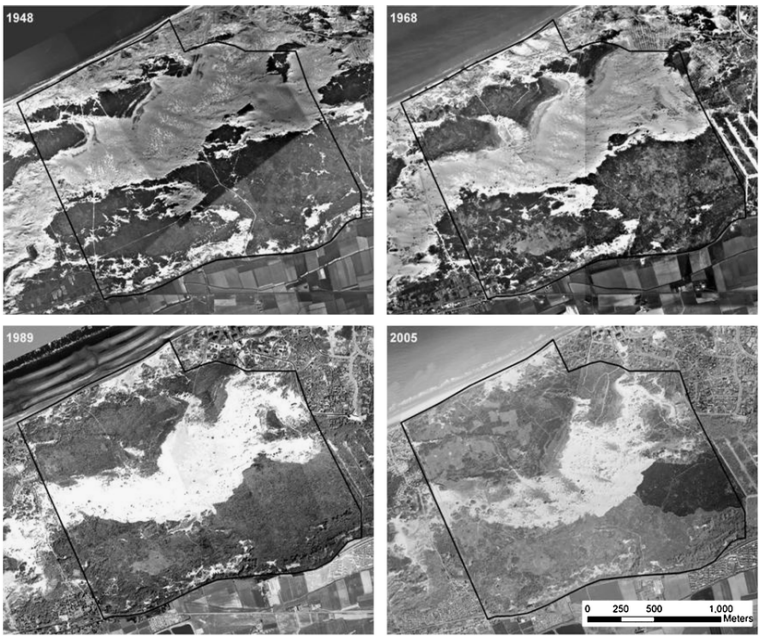


Figure 7.1 Landscape changes in nature reserve 'De Westhoek' in De Panne, Belgium illustrated by a series of aerial photographs. Source: Provoost et al. 2010. Journal of Coastal Conservation.

It thus seems that we are starting to appreciate the complex interactions between the organisms that constitute the above- and belowground community of *A. arenaria* at a time when the very habitat of these species is disappearing at a fast rate. Because some invertebrate species are highly specialised and restricted to *A. arenaria* vegetations, these are expected to be particularly vulnerable. Ironically, some of the most specialised aboveground herbivore species are hardly studied. A striking example is the fact that our main aboveground study species, the aphid *S. rufula*, had not been described from Belgium before the start of our field survey (Vandegehuchte et al. 2010d). This aphid species seems to have a preference for young, vital growing shoots of *A. arenaria* with a dense root system (see chapter 2). On foredunes, *A. arenaria* traps the wind-blown sand, thereby steadily increasing the height of the dune ridge. Therefore, most of the *A. arenaria* tussocks found on these foredunes have grown along with the dune itself, and are tall plants with scattered roots along their vertical rhizomes, which might be of considerable age. Although these plants are vigorous, they

may be less suitable as a host to *S. rufula*. During our field survey, some exceptionally large numbers of aphids were caught on dense, short tussocks, standing more or less solitary in flat areas of bare sand. When the root system was dug out, it appeared that these tussocks had a very heavy clump of roots, without any vertical or horizontal rhizomes. Such tussocks were found in two constructed sea inlets behind the front dune ridge that have recently transformed into bare plains because of sand accretion, but also in the central mobile dunes of the reserve in flat open areas of sand. In some of these flat areas, many thin, short, more or less regularly distributed shoots of *A. arenaria* could be found as well. These shoots also harboured high numbers of *S. rufula* aphids. Although anecdotal and qualitative, these observations are in line with the results of the survey that aphid numbers are positively correlated with root density and shoot vitality and negatively correlated with plant height. These preferred plants seem to have established from seed, given the lack of rhizomes and the short height. Indeed, only in areas where the soil stays damp for a considerable time, seedlings of *A. arenaria* can survive and grow to become mature plants (Huiskes 1979). The former sea inlets and the large open dune slacks are such areas. It therefore appears that seedling establishment, although assumed to be of secondary importance for the reproduction of *A. arenaria*, might be important for *S. rufula*. Since areas suitable for germination are rare (Huiskes 1979) and mainly found in large mobile dune areas where the sand can locally be blown out to the ground water table, *S. rufula* aphids might depend on sand dynamics at a larger scale than its host-plant. Ensuring the presence of such large-scale dynamics might thus be necessary for the conservation of this presumably rare aphid species.

Although root-feeding nematodes have been considered the cause of the decline of *A. arenaria*, it is noteworthy that several species of nematodes are highly specialised feeders of *A. arenaria* roots, such as *Pratylenchus dunensis*, *P. brzeskii* and *Heterodera arenaria* (de la Peña et al. 2007, de la Peña et al. 2008, van der Stoel et al. 2006). These nematode species are replaced by more generalistic root-feeding nematode communities as dunes stabilise, such as *P. penetrans* (de la Peña et al. 2007, de la Peña et al. 2008). Within the habitat of *A. arenaria*, from very dynamic to semi-fixed dunes, we found that root-feeding nematode abundance was higher on plants with a less dense, vital root system. Such fleshy, sparse roots are typically associated

with shoots that are formed along the vertical and horizontal rhizomes of vigorous plants. It thus seems that the fixation of mobile dunes would also disadvantage the specific root-feeding nematode community of *A. arenaria* vegetations, in this case through the reduction of vigorous clonal growth and new rhizome formation.

Mycorrhizal fungi are generally considered to be mutualistic to *A. arenaria*, in the sense that they are able to increase plant biomass and protect the plant from harmful root-feeding nematodes (de la Peña et al. 2006). Yet our results demonstrate that, at least in the seedling stage, AMF can also negatively affect plant performance and as such depress populations of *S. rufula* aphids (see chapter 4). If these aphids indeed prefer young growing shoots in the field, seedlings might constitute an important part of the total resource. If so, mycorrhizal infection of these seedlings might play a role in the distribution of these aphids in the field. The question then follows whether the change in the effect of AMF from parasitic to mutualistic takes place before or after plants have grown beyond their optimal size for the aphids. The abundance of AMF associated with *A. arenaria* is known to be lower on the roots of degenerate stands (Kowalchuk et al. 2002), so depending on this balance, AMF could either compound or mitigate the effects of stabilisation on aphids. A similar experiment as the one we performed using plants of differing age combined with an investigation of the correlation between AMF and aphids in the field, could give a more decisive answer to this question.

Apart from the well-documented decline in *A. arenaria* habitat, our results point at another potential danger to the associated invertebrate community, namely changes in the genotypic composition of *A. arenaria* stands (see chapters 5 and 6). The implications for species conservation are twofold. Firstly, the observation that a majority of invertebrate species clearly differentiated between different plant populations implies that, although *A. arenaria* is common as a species, the conservation of those plant genotypes that are able to support each of the different invertebrate species should be assured. Moreover, *A. arenaria* can be considered a keystone species. It is the dominant plant species in dynamic dunes, often occurring in large monospecific stands, and it is the main cause of sand fixation and as a consequence of the formation of dunes throughout its range. Therefore, changes in the genetic variation of this plant species underlying its associated

biotic interactions might have ecosystem consequences (Whitham et al. 2003). Secondly, *A. arenaria* is often employed to fixate sand, for example in the stabilisation of dikes to protect the hinterland from flooding events (van der Putten 1990). In some cases, massive plantations of this grass are conducted for sand stabilisation, for example in the Dutch dunes of Voorne, where 120 hectares of dune were planted with *A. arenaria* (van der Putten and Kloosterman 1991). This grass is furthermore used as an ornamental species in villages along the coast. Therefore, a scenario comparable to our field setup, in which foreign genotypes are cryptically introduced into local populations of *A. arenaria,* is not unrealistic. Our results demonstrate that most of the aboveground invertebrate species performed better on the local or less distant populations compared to populations from Portugal. If these foreign populations would have the potential to become dominant, for whatever reason, this could be detrimental for the community of aboveground invertebrates. A case in point is the leafhopper *Psammotettix maritimus*, which feeds monophagously on *A. arenaria*. Although all the tested plant populations originate from within this species' range (Ribaut 1952), it clearly preferred the local plant population over all others. Even on plants from the geographically nearest population of Westende, separated by only 15 km from the local population, the occurrence of *P. maritimus* was significantly lower. Note that the plants from De Panne and Westende are significantly differentiated based on neutral molecular markers. This example clearly demonstrates that translocation of *A. arenaria* plants at even very small spatial scales can have immediate impacts on local invertebrate species. The effects on the individual aboveground invertebrate species moreover scale up to the total diversity of the aboveground community. The observation that the diversity of herbivores, predators and detritivores was differently impacted by these non-local genotypes, suggests that trophic relationships between the different species of the community might be altered by these introductions. Analysis of theoretical food webs using logical assumptions based on predator-prey body size ratios indeed confirmed that several statistics of these food webs differed among plant genotypes.

On the other hand, the overall relationship between above- and belowground invertebrate occurrences was negative in our field experiment. This suggests that in the case of *A. arenaria*, a conservation strategy entirely adjusted to preserving those genotypes that favour aboveground

invertebrate species could reduce the performance of some nematode species. Therefore, if the goal of conservation efforts is to promote the entire species community that depends on a keystone plant species, the belowground component of this community is worth considering as well.

Our conclusion that aboveground herbivores are negatively affected by the introduction of non-local plant genotypes, both at the individual species level and at the level of local species richness, raises questions about the role of aboveground herbivores in the invasiveness of *A. arenaria* in other continents, even though we did not quantify the effect of herbivore damage on the performance of the different plant populations. So far, tests of the enemy release hypothesis have been restricted to the belowground natural enemies of *A. arenaria*. This focus on soil organisms is understandable, given the specific and well-studied ecology of *A. arenaria* in its native range, where soil-borne biota are responsible for the decline of the species under stabilising conditions. However, Beckstead and Parker (2003) found that reductions in seed germination, seedling survival and plant growth on non-sterile compared to sterile soil did not differ between the native and introduced range. Knevel et al. (2004) demonstrated that compared to a sterile control soil, *A. arenaria* growth was reduced on soil from 3 out of 5 Dutch locations and from 5 out of 7 South African locations, although on 3 Dutch soils biomass was reduced by half, while this was the case for only one of the South African soils. When grown on natural soils obtained from beneath stands of four different South African dune plant species, *A. arenaria* biomass was significantly reduced on one of the soil types compared to a sterile control soil. The authors conclude from these results that both enemy release and biotic control act on *A. arenaria*, and that the balance between both determines the invasiveness of this species. In a study by van der Putten et al. (2005), the root-feeding nematode fauna of *A. arenaria* was compared between its native range, Europe, and the four major regions where it has been introduced, the USA, South Africa, Australia and New Zealand. The main conclusions were that in most introduced ranges the diversity of root-feeding nematodes was not lower than in Europe, and that both in the native and in the introduced range, *A. arenaria* and related native plants harboured a comparable number of root-feeding nematode species. These results seem to reject the enemy release hypothesis quite convincingly. However, based on the observation that in the regions in the

southern hemisphere less sedentary endoparasitic nematode taxa were found, the authors conclude that introduced *A. arenaria* escapes from specialised enemies, except in the USA. Two aspects of this conclusion are problematic. Firstly, not all sedentary endoparasitic nematode species are equally specialised, some of them are quite generalistic when it comes to host range, and not all nematodes were identified to species level. For example, van der Stoel and van der Putten (2006) found cysts of the cyst nematode *H. hordecalis* in the root zone soil of degenerate *A. arenaria* stands. This species, however, is a generalist feeding on a variety of cereals and grasses (Andersson 1974). Secondly, of all the introduced regions, *A. arenaria* is most aggressively invasive in the USA, while for example in South Africa the species seems to be restricted to the areas where it has been originally planted. Furthermore, the number of taxa was averaged within each geographic range. Because in Europe the number of specialist taxa was lower at the margins of the range than in the centre, the authors attribute the lack of difference between Europe and the USA to the lowered statistical power caused by this averaging. However, the authors consider Poland to be the northern margin of the range, while *A. arenaria* is known to occur as far north as latitude 63°N (Huiskes 1979), roughly halfway Sweden. They moreover consider Scotland to be at the centre of the range, while this corresponds to the outer north-western margin of the European range (Huiskes 1979). Thus the question remains why *A. arenaria* is so invasive in the USA, despite the presence of equal numbers of nematode species, including sedentary endoparasites. In summary, convincing evidence for the generality of the ERH in the context of soil biota has not been put forward yet. In this respect, it is somewhat surprising that the role of aboveground herbivores, although diverse and locally abundant, has never been considered in the ecology of this grass. This conclusion holds true for both the native and the introduced ranges. Although we did not investigate *A. arenaria* in the regions where it is invasive, our field experiment demonstrates that introducing new genotypes of *A. arenaria* into local populations can lead to significantly lowered aboveground herbivore abundance and species richness.

To guarantee the conservation of the diverse above- and belowground invertebrate community associated with *A. arenaria* in its native habitat, it is recommended to allocate management efforts to the preservation of large-

scale aeolian processes in coastal dunes. Large-scale sand-drifts are a prerequisite for the maintenance of a diversity of dynamic dune types, both open sandy patches and blowouts suitable for germination and places with high levels of sand accretion, where *A. arenaria* can continue to grow clonally without losing vigour. In addition, it seems advisable to use local *A. arenaria* plants to obtain material for plantation, as even introductions from nearby populations can induce changes in the performance and diversity of invertebrates. To assure the conservation of native dune vegetations and their associated communities in regions where *A. arenaria* is introduced, understanding the mechanisms which render this species so invasive would be a logical first step. Since release from belowground enemies seems unlikely to explain the exponential spread of *A. arenaria* in some regions, research on the aboveground herbivores in the introduced ranges might yield new insights into the invasive character of *A. arenaria*.

7.3 Implications for the study of aboveground-belowground interactions

Most of the studies on the interactions between above- and belowground herbivores cited in the introduction were conducted in controlled environments. Therefore, the ecological relevance of plant-mediated interactions between root and shoot feeders in complex communities is not well understood. Our study of the root-feeding nematodes and aphids of *A. arenaria* (see chapter 2) demonstrates that significant interactions between above- and belowground herbivores in a laboratory setup, with all other abiotic and biotic variation cancelled out or held constant, do not necessarily lead to detectable patterns in a field situation.

Only a few other studies have investigated patterns of correlation between above- and belowground herbivores in the field. Kaplan et al. (2009) studied the interactions between shoot-feeding insects and root-feeding nematodes of tobacco. To determine the effect of chewing and sucking leaf feeders on nematodes, plants in the field were subjected to either no herbivory, *Manduca sexta* caterpillars, *Myzus persicae* aphids or

both. To investigate the reciprocal interaction, tobacco plants inoculated with *Meloidogyne incognita* nematodes were compared to control plants in a field experiment. Finally, natural occurrences of both shoot-feeding insects and root-feeding nematodes were assessed in the field. Nematodes were positively associated with leaf chewers, but negatively with sap suckers in the field survey. These patterns were relatively stronger than in the manipulative experiments, but similar, thereby confirming that the interactions indeed lead to patterns in the distribution of root and shoot feeders in the field. Kaplan et al. (2009) rightly state that 'testing whether such above- and belowground linkages can be discerned under realistic field conditions despite widespread heterogeneity in biotic and abiotic variables, is a critical step in this emerging ecological field'. The fact that in this study strong patterns were found under field conditions is probably due to the fact that tobacco is a notoriously toxic plant species, where the defence substance in question, nicotine, is exclusively produced in the roots and transported aboveground. Although this study is interesting from an agricultural point of view, crops are known to suffer unnaturally high levels of herbivory due to additional fertilization and extreme densities of host plants (White 1978). The question thus remains whether findings about linkages between root and shoot feeders in these systems, even in the field, can be extrapolated to the natural world.

One of the few field studies using a wild plant species, is that on the interactions between the root-feeding aphid *Pemphigus betae* and the shoot-feeding aphid *Hayhurstia atriplicis* on *Chenopodium album* (Moran and Whitham 1990). In this study, the authors combined experimental approaches with field censuses. In one field experiment, caged plants were placed in the field and inoculated with either or both aphid species to assess their interactions. In another field experiment plants were not caged, and only inoculated with the shoot-feeding aphid, to assess the effect on naturally colonising root-feeding aphids. Finally, plants were destructively sampled in the field to assess the abundances of both root and shoot feeders. In both experiments, plants susceptible as well as resistant to galling by *H. atriplicis* were used. In this study, shoot feeders had a drastic negative effect on root feeders, even leading to local extinctions of the latter. On resistant plants, however, no galls were formed and *P. betae* aphids were not affected. In the field censuses, the root-feeding aphid showed significantly

lower abundances on plants where the shoot-feeding species was present. The effect of *H. atriplicis* on *P. betae* observed in the experiments thus appears to play a role in the distribution and abundance of *P. betae* on naturally occurring host plants. A potential explanation for this field correlation is the magnitude of the effect. The probable reason for the severity of this effect is that both species directly feed on the same source, i.e. the vascular sap, and that leaf gallers are known to divert nutrients within a plant, creating a local nutrient sink.

Our study demonstrates that the agreement between controlled environment and field data does not hold for all types of aboveground-belowground herbivore interactions. In the case of root-feeding nematodes and aphids of *A. arenaria*, the significant reciprocal interactions observed in the laboratory did not translate into correlated abundances in the field, where other environmental factors seemed to have larger effects on plant quality than the presence of herbivores at the other side of the soil surface. We hypothesise that the relative importance of such aboveground-belowground interactions in determining the distributions of the interacting species in the field depends on several aspects of the study system. This importance can be expected to increase for plants i) with high levels of chemical defence substances, ii) with high natural abundances of above- and belowground herbivores, especially when they both feed on phloem, iii) growing in temporally and spatially homogeneous habitats.

One of the potential factors that might affect the interactions between above- and belowground organisms, is the abiotic composition of the soil. In our experiment (chapter 3) we have demonstrated that the relative importance of biotic and abiotic soil properties to plants and their aboveground herbivores can differ. Our finding that one effect of soil biota was only apparent in one of three geographically separated dune regions, while another held true across these regions, highlights the importance of replication across space to make inferences about the generality of aboveground-belowground interactions. In a sense this relates to the previous paragraphs about the testing of these interactions in the field. Interactions between above- and belowground organisms can depend not only on the particular species involved, but also on the local environment. Spatial replication of experiments or surveys across landscapes provides a means of discovering such context dependency. Moreover, additional study

of the relative importance of biotic and abiotic soil components in aboveground-belowground ecology is clearly needed. Our simple cross-inoculation methodology could be employed to address this question in many different systems.

As detailed in the introduction, the effects of intraspecific genetic variation in plants on the communities and ecosystems to which these plants belong, the so-called extended phenotypes, have been demonstrated for a number of plant systems. Also the patterns of covariation between different species of the community in their association with plant genotypes have been amply investigated over the past decades. However, this considerable body of literature has almost entirely focused on what occurs aboveground, thereby ignoring an entire community living in connection with the roots of plants. The results of our field experiment (chapter 5) demonstrate that the consideration of this subterranean community drastically alters our conclusions about the effects of plant genotypic variation on the associated community. If our study had focused only on the above- or belowground community, we would have reached the same conclusion, namely that the responses of the invertebrate species towards plant genotypes are positively correlated. This conclusion would have led to the idea that certain plant genotypes are characterised by some generalised defence mechanism that is effective against a range of species, or by some aspect of plant quality that attracts different invertebrate species. However, the negative correlation between above- and belowground invertebrate species revealed that there might be a genetically embedded trade-off between the plant's susceptibility to above- and belowground invertebrate species. One of the questions in the study of plant-herbivore interactions is why variation in resistance levels is maintained in plants. Why aren't plants evolving towards total herbivore resistance, if herbivore damage results in lower plant fitness? A common explanation for this phenomenon is that there are costs associated with resistance. A cost of resistance to enemies is apparent when resistant plants perform worse than susceptible plants in the absence of those enemies. Costs of resistance can be divided into direct and indirect, or ecological costs. A direct resistance cost is a trade-off between fitness and resistance that is not mediated by interactions with other species, as is the case with indirect resistance costs. Direct costs can arise when a secondary plant chemical is not only toxic to herbivores, but also to the plant itself, or when the

resistance trait allocates resources away from other plant functions, such as photosynthesis. There is a huge diversity of potential ecological costs, including the deterrence of mutualists and the increased impact of other herbivores. Resistance pathways against different enemies might compete for shared precursors and not be fully deployed simultaneously. On the other hand, the same plant trait that deters one herbivore might attract others, particularly specialists. In a review on the costs of resistance to herbivory, Strauss et al. (2002) list the assessment of the prevalence and magnitude of ecological costs of resistance as one of the major challenges in this field. In their review, Strauss et al. (2002) detected costs of resistance in 76% of the studies examined, a markedly higher proportion than the 33% in a previous review of this subject (Bergelson and Purrington 1996). However, this still implies that in roughly one quarter of the included studies, no costs could be detected, although variation in resistance was observed. Our results suggest that the ecological cost of increased resistance to aboveground herbivores in *A. arenaria* might be an increased susceptibility to root-feeding nematodes. This trade-off could be caused by opposing effects of a same genetically determined plant trait, or by the earlier arriving aboveground herbivores causing a plant-mediated negative effect on the abundance of the nematodes. However, this does not alter our conclusion, since the resulting cost of the resistance to aboveground herbivores is a higher susceptibility to root feeders in both cases. Interestingly, the earlier discussed study of Moran and Whitham (1990) demonstrated a similar trade-off, in which the resistance to aboveground leaf-galling aphids caused an increased susceptibility of the roots to root-feeding aphids. Study in other systems will have to determine whether negative relationships between above- and belowground herbivores are a general phenomenon.

7.4 Future perspectives and opportunities

The dune grass *A. arenaria* has been a model species for the study of plant-soil feedbacks for several decades. We have taken advantage of this ecological knowledge to develop *A. arenaria* into a model system for addressing some of the issues that recently have arisen in the field of aboveground-belowground ecology. As we ventured out of the soil into the

aerial part of this particular system, we have exposed some new and exciting patterns and interactions. However, as is often the case when breaking new ground, each answered question raised a multiple of new ones. We are fully aware that many of the implications of our results, proposed in this chapter so far, are to a large extent hypothetical. Therefore, the aim of this last section is to outline some interesting avenues for future research to test these hypotheses or to investigate the yet unexplained.

We have demonstrated different interactions between the above- and belowground biota of the *A. arenaria* model system as well as effects of variation within *A. arenaria* on both these groups of organisms. Our research has often focused on effects rather than on mechanisms. A straightforward reason why we did not actively pursue the mechanistic basis of the observed interactions and patterns is the bewildering multitude of potential plant traits that can act as mediators of these effects. Our first priority was to verify whether aboveground-belowground interactions and effects of plant genotypic variation were possible, and if so, to determine their direction and magnitude. However, a next crucial step would be to identify the particular plant traits responsible for these observations. The fact that we dispose of different populations of *A. arenaria* with known differences in their effects on above- and belowground invertebrates might facilitate this identification process. Plant traits that quantitatively differ between these populations, whose trait levels correlate with the magnitudes of effects on certain invertebrates, could be prioritised to investigate experimentally. This way, traits that consistently differ between populations could be identified. Of course, to unravel the mechanism of the interactions between mycorrhizal fungi or root-feeding nematodes and aphids, plants should be experimentally subjected to these organisms and the traits of interest should be compared with a control group of plants. It would be informative to determine how levels of nutrients, especially essential amino acids, change in leaves and roots in response to root or shoot herbivory or mycorrhizal colonisation. Although currently no chemical defence molecules have been described from *A. arenaria*, a range of secondary metabolites with a known defence function have been found in other grass species (reviewed in Sanchez-Moreiras et al. 2003). Screening of *A. arenaria* for these substances might thus reveal to what extent this species has the potential for chemical defence. Although some endophytic fungi are known to provide grasses with alkaloids, and

Acremonium species have been described from *A. arenaria* (Hol et al. 2007), seeds of the populations we used in the experiments have been screened for endophytes with no result so far, except for a very low percentage of seeds from the Oostvoorne population (M. Omacini and E. de la Peña, unpublished result). This renders mechanisms involving endophytic fungi unlikely in our case. Another element that can lead to a range of known direct and indirect physical and chemical defence properties in plants, is silicon (Reynolds et al. 2009). The fact that Si has proven an effective feeding deterrent against a range of herbivore feeding guilds, makes this element particularly interesting as a candidate mechanism for our observations. Furthermore, it would be particularly interesting to investigate which traits might be responsible for the observed negative relationship between above- and belowground invertebrates across the used plant genotypes. It could be that specialised shoot herbivores induce a general defence response in the plant, to which they have evolved resistance, but the root feeders are still sensitive. Another option is that plants who allocate a larger proportion of their primary nutrients to leaves are more attractive to shoot feeders and less preferred by root feeders.

As mentioned in the previous paragraph, the patterns of covariation among above- and belowground invertebrates colonising the different plant genotypes could be based on ecological interactions. Indeed, the presence of one species can affect the subsequent selection of host-plants by other species. The multitude of possible interactions between above- and belowground invertebrates moreover imply that selection might also be based on the presence of other species at the other side of the soil surface. Therefore, our results do not allow to determine whether the association with each of the species is based directly on genetically determined plant traits or indirectly through the consequences of these traits on the interactions with other invertebrate species. It is important to note that truly genetic correlations, as well as correlations based on one species' presence affecting the fitness effects of other species, exclude the possibility of pairwise coevolution (Hougen-Eitzman and Rausher 1994, Inouye and Stinchcombe 2001, Iwao and Rausher 1997, Stinchcombe and Rausher 2001). The only way to distinguish between these mechanisms, is by testing the individual response of each species to the different plant genotypes in the absence of other species, and then correlating these independent responses.

In a system with two invertebrate species, this can be feasible. To experimentally test each of thirteen invertebrate species in isolation becomes very difficult. Future experiments in the *A. arenaria* system could select a small subset of the species we tested in chapter 5. Given our observation that above- and belowground invertebrate species formed negatively covarying clusters, it might also be worthwhile to perform an experiment in which either the above- or belowground invertebrates are excluded. This could already establish whether root-feeding nematodes chose genotypes based on the presence of the earlier arriving aboveground invertebrates, or whether this preference is inherently linked to the genetic composition of the plants. In their meta-analysis, Leimu and Koricheva (2006) found that genetic correlations in studies where resistances were assessed in the presence of other natural enemies were lower than when resistances were quantified independently. This implies that interactions between different enemies in the field are predominantly negative and tend to reduce the magnitude of correlations. The considerable magnitude of the correlations observed in our study would thus likely have been even higher if we had measured associations with each invertebrate species independently.

In order to know whether the observed associations with different invertebrate species have implications for the evolution of *A. arenaria*, it is necessary to quantify the effects of these invertebrates on plant fitness. All of the recorded root-feeding nematode genera have proven to reduce biomass at least in some cases. The aphid *S. rufula* furthermore drastically reduces plant performance under laboratory conditions (see chapters 2, 3 and 4). Although these findings suggest that fitness effects of several of the involved invertebrate species are highly plausible, this still has to be demonstrated. Because our plants did not flower in the field, quantifying fitness is not straightforward. *A. arenaria* plants established from seeds need two and usually more years before they start flowering (Huiskes 1979). Moreover, as this species reproduces mainly by clonal spread, the relative amount of viable seeds might not be the best measure of fitness. Quantifying the effects of all involved invertebrate species on plant biomass could provide a first proxy for fitness effects. Another improvement to our experiment could be made by using a design that allows to estimate narrow-sense genetic variation in associations of *A. arenaria* with different invertebrate species. Because this plant is strongly protandrous, autogamy

does not occur (Huiskes 1979), simplifying the specific crosses needed to obtain pedigree classes suited for quantitative genetic analysis.

Although our field experiment has demonstrated that introduced genotypes of *A. arenaria* affected the responses of both the above- and belowground invertebrate community, we do not know whether any of these patterns are a consequence of local adaptation or maladaptation. It would therefore be interesting to repeat the experiment with the same plant populations in the locations of origin of each of these populations. For example, it could be that in Comporta local aboveground herbivorous invertebrates prefer Comporta plants over those from other populations. This would imply that Comporta plants are not inherently better defended against herbivory than plants from De Panne, even though in De Panne they were less preferred by aboveground herbivores. In this respect, it could also be informative to reciprocally transplant populations between Europe and the introduced ranges of *A. arenaria*. If such transplantations would be combined with selective above- and belowground herbivore exclusions, much knowledge could be gathered about the respective roles of either type of herbivore in the invasiveness of this grass species.

Our results have demonstrated that genotypes of *A. arenaria* are characterised by distinctive above- and belowground invertebrate communities, or so-called community phenotypes. A next step would be to find out whether these community phenotypes in turn lead to ecosystem phenotypes. For example, one of the invertebrate species that was strongly affected by *A. arenaria* genotype, was the detritivore *P. scaber*. If this detritivore contributes significantly to decomposition, this suggests that genotypic variation in *A. arenaria* might cause variation in decomposition rates which in turn might affect ecosystem processes such as nitrogen mineralisation. On the other hand, such ecosystem phenotypes could also directly follow from genetically based traits of *A. arenaria* which render its leaf litter more or less degradable to the microbial community. Such hypotheses could for example be tested with experiments using litter bags with litter from different genotypes.

We have tried to develop *A. arenaria* into a model system for the research on aboveground-belowground interactions. Using this model system, we have explored some of the aspects of this field of ecology that

had received little attention so far. The work we have presented here gives evidence of the complex and exciting processes taking place in the ecological community centred around *A. arenaria*, crossing the boundary between air and soil. However, this last section has pointed out that these discoveries only mark the beginning of our understanding of this intricate matter. Several exciting issues are yet to be resolved, especially the role of abiotic factors in the interactions between above- and belowground biota, the evolutionary consequences of these interactions and the role of plant genetic variation in the structuring of both above- and belowground communities and ecosystems.

8 Summary

For a long time, the major part of ecological theory has been based on the observations made by aquatic and aboveground ecologists, while soil biologists have been furthering their field largely ignoring what went on above the soil surface. A few decades ago, however, ecologists started to realise that plants enable interactions and feedbacks between the above- and belowground components of ecosystems. Since then, a plethora of interactions, positive as well as negative, between above- and belowground herbivores, detritivores, mutualists and even higher trophic levels have been documented. These discoveries have demonstrated the importance of aboveground-belowground interactions in the structuring of communities and functioning of ecosystems.

Although the study of aboveground-belowground interactions has become a fast-expanding field in ecology, several aspects of these interactions remain largely unexplored. So far, studies have mostly focused on the interactions between biota at either side of the soil surface. How the abiotic soil component impinges upon these interactions, has hardly been addressed experimentally. Furthermore, aboveground-belowground linkages have mainly been studied from a purely ecological perspective. Very little is currently known about the evolutionary consequences of these linkages for the organisms that make up the community. Finally, interactions between root- and shoot-associated organisms are still mainly investigated by means of controlled laboratory experiments using a small number of interacting species. Whether conclusions based on these studies hold in a realistic context of multiple interacting species and abiotic factors at both sides of the surface, is still to be elucidated.

In an attempt to narrow some of these gaps in our knowledge, this thesis focuses on the dune grass *Ammophila arenaria*. This species forms one of the best documented model systems for plant-soil interactions. *A. arenaria* only thrives in dynamic dunes with a high rate of sand accretion. As soon as the dunes start to stabilise, this grass loses its vigour and starts to degenerate, making way for later successional species. This phenomenon has been called

the '*Ammophila* problem', and over the past century, many hypotheses have been put forward about its cause. Earlier explanations for the decline in the absence of wind-blown sand include a lack of nutrients, an age effect, poor aeration of roots and rhizomes, toxic secretion substances, increased competition from other plant species and an inefficient replacement of old roots. However, a little over two decades ago, it was discovered that growth of *A. arenaria* could be improved by sterilising the rhizosphere soil, indicating the presence of a growth reducing biotic soil factor. This led to a new hypothesis, namely that *A. arenaria* needs a continual supply of fresh sand to grow upwards and escape from the accumulating root pathogens. Further research demonstrated that this negative effect is the net outcome of complex rhizosphere interactions involving root antagonists such as pathogenic fungi and nematodes, root mutualists such as mycorrhizal fungi and organisms able of controlling the antagonists, such as other nematodes, microorganisms and microarthropods. However, in recent years the original hypothesis of root efficiency of nutrient extraction has revived. In contrast to the large body of literature on the interactions in the rhizosphere of *A. arenaria*, stands the very scarce information on the ecology of its diverse aboveground fauna. This thesis investigates the role of these shoot-associated organisms in the ecology of the community centred around *A. arenaria*, and by doing so tries to address some of the general questions in current aboveground-belowground ecology.

In chapter 2, interactions between root-feeding nematodes and the specialist aphid *Schizaphis rufula* in the laboratory were compared with patterns of abundance in the field. Under controlled conditions, aphids negatively affected nematodes and vice versa. In the field, however, no significant correlation between aphid and nematode abundances could be detected. There, aphid numbers were highest on plants with short, vigorous shoots and a dense root system, while nematodes were most numerous on plants with a less dense, vital root system. Caution should thus be exerted when extrapolating findings from laboratory experiments to the field situation, where other biotic and abiotic variables might be relatively more important for herbivore populations than the presence of other herbivores at the other side of the soil surface.

In chapter 3, we investigated the performance of *A. arenaria* seedlings and *S. rufula* aphids on sterile soils from dynamic and stabilised dunes and on

those same soils cross-inoculated with biota of either dune type, in order to disentangle the relative importance of the biotic and abiotic soil component. This setup was, moreover, replicated across three spatially separated dune systems. Plants were mostly affected by biota, in accordance with the escape hypothesis, although not all effects were detectable across the three dune areas. Aphids, on the other hand, only reacted to abiotic soil differences between the three dune regions. These findings demonstrate that plants and their insect herbivores can be differently affected by different aspects of the soil. Furthermore, they highlight the context dependency of some interactions and the need for spatial replication.

In chapter 4, a negative effect of arbuscular mycorrhizal fungi (AMF) on *A. arenaria* seedlings and *S. rufula* aphids was demonstrated. The effect of AMF on the aphids was likely to be mediated by the observed decrease in plant biomass, since only the final population size of the aphids was affected, and not the individual development time or population growth rate. Moreover, when including shoot biomass as a covariate in the model, the effect of AMF on aphids disappeared. Therefore, total plant biomass presents a straightforward mechanism by which AMF can affect insect herbivores. Such effects, however, remain undetected by studying the performance of insects on detached individual leaves or excised leaf discs. Since AMF have previously been shown to benefit the growth of *A. arenaria*, the observed negative effect in this study might be due to the young age of the seedlings.

In chapters 5 and 6, an experiment is discussed in which different genotypes of *A. arenaria*, originating from different locations within Europe, were exposed to natural colonisation by above- and belowground invertebrates in a common garden. Plant genotype affected the occurrence of different species of invertebrates. Strong positive covariation across genotypes was detected among the aboveground as well as among the belowground invertebrate species' occurrences. However, correlations across genotypes between above- and belowground invertebrates were on average negative, suggesting a trade-off between the susceptibility of *A. arenaria* to root- and shoot-associated species. Moreover, the species richness of the aboveground fauna was significantly lower on several of the introduced plant genotypes compared to the local genotype, especially that of herbivorous invertebrates. This depauperate species richness led to an

altered food web structure, characterised by a lower number of links per species.

The results presented in this thesis hold several implications, both for nature conservation and the general ecology of aboveground-belowground interactions. The mobile sand dunes where *A. arenaria* thrives, are threatened in their existence throughout Europe. Nematodes and aphids in the field reached their highest abundance on different types of *A. arenaria* plants, growing optimally in different habitats within the dynamic dune landscape. The preservation of such a diverse dune landscape is only possible when the presence of large-scale sand dynamics is assured. Since *A. arenaria* is planted throughout Europe for sand stabilisation, a scenario in which non-local genotypes are introduced into local populations is realistic. Caution should be exerted when selecting material for such plantations, given the observation that introduced populations can drastically lower the diversity of the aboveground invertebrate community. On the other hand, given the overall negative correlation between and above- and belowground invertebrate species across genotypes, conserving only those genotypes that optimise the aboveground diversity might have negative consequences for the conservation of the belowground community. *A. arenaria* has also been introduced in other continents for erosion control, and has become highly invasive in several parts of its new range. Explanations have, so far, been sought in the release of belowground enemies. The result that introduced populations supported significantly less aboveground herbivore species in the common garden, however, raises questions on the role of aboveground enemies in the invasiveness of this grass. The high degree of covariation among the responses of different invertebrate species to plant genotype suggests that coevolution between *A. arenaria* and its associated fauna is diffuse rather than pairwise. In addition, the possibility of a trade-off between the resistance of *A. arenaria* to above- and belowground invertebrates sheds new light on the question why several studies fail to detect any cost of resistance. Such costs are a common explanation for the maintenance of variable levels of resistance. If the ecological cost of increased resistance to aboveground enemies is a decreased resistance to belowground enemies, such cost will go unnoticed in studies ignoring the belowground component of the ecosystem.

Although the results presented in this thesis provide a first step towards a more integrated understanding of the aboveground-belowground interactions in the *A. arenaria* system, many questions remain to be answered. For example, the particular plant traits mediating the observed effects and interactions still have to be identified. Exclusion experiments could elucidate whether responses of invertebrates to plant genotypes are due to traits induced by other, earlier arriving invertebrates or to genetically determined constitutive plant traits. Reciprocal transplantation experiments could further assess to what extent the observed patterns are a consequence of local adaptation. Finally, quantifying the effects of different invertebrate species on plant fitness could yield insight into the consequences of the observed interactions for the evolution of *A. arenaria*.

9 Samenvatting

Gedurende lange tijd is het merendeel van de ecologische theorie gebaseerd op observaties van bovengrondse en aquatische systemen. Intussen ontwikkelden bodembiologen hun eigen veld, waarbij ze grotendeels negeerden wat zich afspeelde boven het bodemoppervlak. Enkele decennia geleden begonnen ecologen zich echter te realiseren dat planten interacties mogelijk maken tussen de boven- en ondergrondse component van ecosystemen. Sindsdien is een veelheid aan interacties, zowel positieve als negatieve, tussen boven- en ondergrondse herbivoren, detritivoren, mutualisten en zelfs hogere trofische niveaus gedocumenteerd. Deze ontdekkingen hebben het belang aangetoond van bovengronds-ondergrondse interacties voor het structureren van gemeenschappen en het functioneren van ecosystemen.

Hoewel de studie van bovengronds-ondergrondse interacties een snel groeiende tak van de ecologie geworden is, zijn meerdere aspecten van deze interacties nog grotendeels onbekend. Tot hiertoe hebben studies zich voornamelijk toegespitst op de interacties tussen biota aan weerszijden van het bodemoppervlak. Hoe de abiotische bodemcomponent inwerkt op deze interacties, is nog amper experimenteel onderzocht. Voorts zijn koppelingen tussen het onder- en bovengrondse hoofdzakelijk bestudeerd vanuit een puur ecologisch perspectief. Zeer weinig is momenteel geweten over de evolutionaire gevolgen van deze interacties voor de organismen die deel uitmaken van de ecologische gemeenschap. Ten slotte worden interacties tussen organismen die geassocieerd zijn met wortels en scheuten nog steeds in hoofdzaak onderzocht door middel van gecontroleerde laboratorium-experimenten met een klein aantal interagerende soorten. Of conclusies gebaseerd op dergelijke studies stand houden in een realistische context met meerdere interagerende soorten en abiotische factoren aan weerszijden van het oppervlak, dient nog opgehelderd te worden.

In een poging om enkele van deze hiaten in onze kennis te dichten, concentreert dit proefschrift zich op het duingras helm, *Ammophila arenaria*. Deze soort vormt een van de best gedocumenteerde modelsystemen voor

interacties tussen plant en bodem. *A. arenaria* gedijt enkel in dynamische duinen met een hoge mate van zandaanwas. Zodra de duinen beginnen te stabiliseren, verliest dit gras zijn vitaliteit en begint het te degenereren. Het wordt dan vervangen door de volgende soorten in de successie. Dit fenomeen werd het '*Ammophila* probleem' genoemd en over de afgelopen eeuw werden vele hypotheses naar voren geschoven over de oorzaak ervan. Vroegere verklaringen voor de achteruitgang in de afwezigheid van aangewaaid zand omvatten een gebrek aan nutriënten, een verouderingseffect, slechte verluchting van wortels en rhizomen, toxische secretieproducten, een toegenomen competitie door andere plantensoorten en een inefficiënte vervanging van oude wortels. Iets meer dan twee decennia geleden ontdekte men echter dat de groei van *A. arenaria* verbeterd kon worden door de bodem te steriliseren. Dit wees op de aanwezigheid van een groei reducerende, biotische bodemfactor. Een nieuwe hypothese luidde dat *A. arenaria* een continue aanvoer van vers zand nodig heeft om omhoog te kunnen groeien en zo aan de zich opstapelende bodempathogenen te kunnen ontsnappen. Verder onderzoek toonde aan dat dit negatieve effect de netto uitkomst is van complexe interacties in the rhizosfeer, tussen antagonisten zoals pathogene schimmels en nematoden, mutualisten zoals mycorrhizaschimmels, en organismen die de antagonisten kunnen controleren, zoals andere nematoden, micro-organismen en microarthropoden. In de afgelopen jaren leefde de oorspronkelijke hypothese over de efficiëntie van nutriëntenextractie door wortels echter terug op. In tegenstelling tot de aanzienlijke hoeveelheid literatuur over de interacties in de rhizosfeer van *A. arenaria*, is zeer weinig informatie bekend over de ecologie van de diverse bovengrondse fauna die met dit gras geassocieerd is. Deze thesis onderzoekt de rol van deze bovengrondse organismen in de ecologie van de gemeenschap rondom *A. arenaria* en probeert zo enkele van de algemene vragen in de bovengronds-ondergrondse ecologie te beantwoorden.

In hoofdstuk 2 werden interacties tussen worteletende nematoden en de gespecialiseerde bladluis *Schizaphis rufula* in het laboratorium vergeleken met patronen van abundantie in het veld. Onder gecontroleerde omstandigheden hadden de bladluizen een negatief effect op de nematoden en omgekeerd. In het veld kon echter geen significante correlatie vastgesteld worden tussen de abundantie van bladluizen en nematoden. Hier was het

aantal bladluizen het hoogst op planten met korte, vitale scheuten en een hoge densiteit aan wortels. Nematoden waren het talrijkst op planten met een lagere densiteit aan vitale wortels. Voorzichtigheid is dus geboden bij het extrapoleren van bevindingen van het laboratorium naar het veld waar andere biotische en abiotische variabelen relatief belangrijker kunnen zijn voor herbivorenpopulaties dan de aanwezigheid van andere herbivoren aan de andere zijde van het bodemoppervlak.

In hoofdstuk 3 onderzochten we de groei van *A. arenaria* zaailingen en de dynamiek van *S. rufula* populaties op steriele bodems van dynamische en gestabiliseerde duinen en op diezelfde bodems, geïnoculeerd met biota van het eigen of het andere duintype. Op die manier kon het relatieve belang van de biotische en abiotische bodemcomponent bepaald worden. Deze opstelling werd bovendien gerepliceerd over drie ruimtelijk gescheiden duingebieden. Planten waren hoofdzakelijk beïnvloed door de biotische component, in overeenstemming met de ontsnappingshypothese, hoewel niet alle effecten detecteerbaar waren voor elk van de drie duingebieden. De bladluizen daarentegen reageerden enkel op abiotische verschillen tussen bodems van de drie duingebieden. Deze bevindingen tonen aan dat planten en hun herbivore insecten verschillend beïnvloed kunnen worden door verschillende aspecten van de bodem. Bovendien benadrukken ze de contextafhankelijkheid van sommige interacties en de nood aan ruimtelijke replicatie.

In hoofdstuk 4 werd een negatief effect van arbusculaire mycorrhiza-schimmels (AMS) op zaalingen van *A. arenaria* en *S. rufula* bladluizen aangetoond. Het effect van AMS op de bladluizen was vermoedelijk te wijten aan de geobserveerde vermindering in biomassa van de plant, aangezien enkel de finale populatiegrootte van de bladluizen beïnvloed werd, en niet de individuele ontwikkelingstijd of groeisnelheid van de populatie. Bovendien, wanneer biomassa als een covariaat in het model opgenomen werd, verdween het effect van AMS op de bladluizen. De biomassa van de gehele plant biedt dus een eenvoudig mechanisme waarlangs AMS herbivore insecten kunnen beïnvloeden. Dergelijke effecten worden echter niet gedetecteerd door het bestuderen van de prestaties van insecten op individuele, losgemaakte bladeren. Aangezien AMS in voorgaande studies de groei van *A. arenaria* bevorderden, zou het hier geobserveerde negatieve effect te wijten kunnen zijn aan de jonge leeftijd van de zaailingen.

In hoofdstuk 5 en 6 werd een experiment besproken waarin verschillende genotypes van *A. arenaria*, afkomstig van verschillende locaties in Europa, blootgesteld werden aan natuurlijke kolonisatie door boven- en ondergrondse invertebraten in een gemeenschappelijke omgeving. Plantgenotype beïnvloedde het voorkomen van verschillende invertebratensoorten. Een sterke positieve covariatie werd vastgesteld zowel tussen de ondergrondse als tussen de bovengrondse invertebraten in hun voorkomen over de genotypes heen. Correlaties tussen boven- en ondergrondse invertebraten over de genotypes heen waren gemiddeld negatief, wat een balans suggereert tussen de vatbaarheid van *A. arenaria* voor soorten die geassocieerd zijn met wortels en scheuten. Bovendien was de soortenrijkdom van de bovengrondse fauna, en in het bijzonder deze van de herbivoren, significant lager op verschillende van de geïntroduceerde plantgenotypes in vergelijking met het lokale genotype. Deze verarmde soortenrijkdom leidde tot een veranderde voedselwebstructuur, gekenmerkt door een lager aantal links per soort.

De resultaten die gepresenteerd worden in deze thesis hebben verschillende implicaties, zowel voor natuurbehoud als voor de algemene ecologie van bovengronds-ondergrondse interacties. De mobiele zandduinen waar *A. arenaria* gedijt, worden bedreigd in hun voortbestaan op Europese schaal. Nematoden en bladluizen bereikten in het veld hun hoogste dichtheden op verschillende types *A. arenaria*, die optimaal groeien in verschillende habitats binnen het dynamische duinlandschap. Het behoud van een dergelijk divers duinlandschap is enkel mogelijk wanneer de aanwezigheid van zanddynamiek op grote schaal gevrijwaard wordt. Aangezien *A. arenaria* in heel Europa aangeplant wordt voor zandstabilisatie, is een scenario waarin niet-lokale genotypes geïntroduceerd worden in lokale populaties realistisch. Voorzichtigheid is geboden bij het selecteren van materiaal voor dergelijke aanplanten, gezien de observatie dat geïntroduceerde genotypes de diversiteit van de bovengrondse invertebratengemeenschap drastisch kunnen reduceren. Anderzijds, gezien de gemiddeld negatieve correlatie tussen boven- en ondergrondse invertebraten over plantgenotypes heen, kan het behouden van enkel die genotypes die de bovengrondse diversiteit optimaliseren negatieve gevolgen hebben voor het behoud van de ondergrondse gemeenschap. *A. arenaria* werd bovendien ook geïntroduceerd in andere continenten in het kader van

erosiebestrijding, en is zeer invasief geworden in verschillende van deze nieuwe gebieden. Verklaringen hiervoor werden tot hiertoe gezocht in een bevrijding van ondergrondse vijanden. Het resultaat dat op geïntroduceerde populaties significant minder soorten bovengrondse herbivoren aangetroffen werden, roept echter vragen op over de rol van bovengrondse vijanden in het invasief karakter van dit gras. De hoge mate van covariatie tussen de reacties van verschillende invertebratensoorten op plantgenotype suggereert dat co-evolutie tussen *A. arenaria* en de geassocieerde fauna diffuus en niet paarsgewijs verloopt. Daarenboven werpt de mogelijkheid van een balans tussen de resistentie van *A. arenaria* tegen boven- en ondergrondse invertebraten een nieuw licht op de vraag waarom verschillende studies er niet in slagen om een kost verbonden aan resistentie te detecteren. Dergelijke kosten worden gewoonlijk aangehaald als verklaring voor het blijven bestaan van variabele resistentieniveaus. Indien de ecologische kost van een toegenomen resistentie tegen bovengrondse vijanden een afname in resistentie tegen ondergrondse vijanden is, zullen dergelijke kosten niet opgemerkt worden in studies die de ondergrondse component van het ecosysteem negeren.

Hoewel de resultaten die gepresenteerd worden in deze thesis een eerste stap vormen in de richting van een meer geïntegreerde kennis van de bovengronds-ondergrondse interacties in het *A. arenaria* systeem, blijven vele vragen nog onbeantwoord. De specifieke plantkenmerken die aan de basis liggen van de geobserveerde effecten en interacties dienen bijvoorbeeld nog geïdentificeerd te worden. Gerichte uitsluitings-experimenten kunnen ophelderen in hoeverre de reacties van invertebraten op plantgenotypes te wijten zijn aan kenmerken geïnduceerd door andere, eerder arriverende invertebraten of aan genetisch bepaalde constitutieve plantkenmerken. Wederzijdse transplantatie-experimenten zouden verder kunnen uitmaken tot op welke hoogte de geobserveerde patronen een gevolg zijn van lokale adaptatie. Ten slotte zou het kwantificeren van de effecten van de verschillende invertebratensoorten op de fitness van de plant inzichten kunnen genereren omtrent de gevolgen van de geobserveerde interacties voor de evolutie van *A. arenaria*.

10 References

Abdel Wahab A. M. 1975. Nitrogen-fixation by *Bacillus* strains isolated from rhizopshere of *Ammophila arenaria*. - Plant and Soil 42: 703-708.

Abdel Wahab A. M. and Wareing P. F. 1980. Nitrogenase activity associated with the rhizosphere of *Ammophila arenaria* L and effect of inoculation of seedlings with *Azotobacter*. - New Phytologist 84: 711-721.

Anderson M. J., Gorley R. N. and Clarke K. R. 2008. Permanova+ for primer: Guide to software and statistical methods. - PRIMER-E, Plymouth, UK.

Andersson S. 1974. *Heterodera hordecalis* n. sp. (nematoda: Heteroderidae) a cyst nematode of cereals and grasses in southern Sweden. - Nematologica 20: 445-&.

Anwar S. A. and McKenry M. V. 2007. Variability in reproduction of four populations of *Meloidogyne incognita* on six cultivars of cotton. - Journal of Nematology 39: 105-110.

Augé R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. - Mycorrhiza 11: 3-42.

Baker G. H. and Vogelzang B. K. 1988. Life-history, population-dynamics and polymorphism of *Theba pisana* (Mollusca, Helicidae) in Australia. - Journal of Applied Ecology 25: 867-887.

Baker T. J. and Gowen S. R. 1996. Staining: Nematodes and arbuscular mycorrhizae in the same root sample. - Fundamental and Applied Nematology 19: 607-608.

Bangert R. K., Turek R. J., Rehill B., Wimp G. M., Schweitzer J. A., Allan G. J., Bailey J. K., Martinsen G. D., Keim P., Lindroth R. L. and Whitham T. G. 2006. A genetic similarity rule determines arthropod community structure. - Molecular Ecology 15: 1379-1391.

Bangert R. K. and Whitham T. G. 2007. Genetic assembly rules and community phenotypes. - Evolutionary Ecology 21: 549-560.

Barbour R. C., Forster L. G., Baker S. C., Steane D. A. and Potts B. M. 2009. Biodiversity consequences of genetic variation in bark characteristics within a foundation tree species. - Conservation Biology 23: 1146-1155.

Bardgett R. D., Cook R., Yeates G. W. and Denton C. S. 1999. The influence of nematodes on below-ground processes in grassland ecosystems. - Plant and Soil 212: 23-33.

Bardgett R. D. and Wardle D. A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. - Ecology 84: 2258-2268.

Barot S., Blouin M., Fontaine S., Jouquet P., Lata J. C. and Mathieu J. 2007. A tale of four stories: Soil ecology, theory, evolution and the publication system. - PLoS ONE 2: e1248.

Beckstead J. and Parker I. M. 2003. Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens? - Ecology 84: 2824-2831.

Bennett A. E., Bever J. D. and Bowers M. D. 2009. Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. - Oecologia 160: 771-779.

Bergelson J. and Purrington C. B. 1996. Surveying patterns in the cost of resistance in plants. - American Naturalist 148: 536-558.

Bever J. D., Westover K. M. and Antonovics J. 1997. Incorporating the soil community into plant population dynamics: The utility of the feedback approach. - Journal of Ecology 85: 561-573.

Bezemer T. M., Wagenaar R., Van Dam N. M. and Wackers F. L. 2003. Interactions between above- and belowground insect herbivores as mediated by the plant defense system. - Oikos 101: 555-562.

Bezemer T. M., De Deyn G. B., Bossinga T. M., Van Dam N. M., Harvey J. A. and Van Der Putten W. H. 2005. Soil community composition drives aboveground plant-herbivore-parasitoid interactions. - Ecology Letters 8: 652-661.

Bezemer T. M. and Van Dam N. M. 2005. Linking aboveground and belowground interactions via induced plant defenses. - Trends in Ecology & Evolution 20: 617-624.

Blossey B. and Hunt-Joshi T. R. 2003. Belowground herbivory by insects: Influence on plants and aboveground herbivores. - Annual Review of Entomology 48: 521-547.

Boag B. and Yeates G. W. 1998. Soil nematode biodiversity in terrestrial ecosystems. - Biodiversity and Conservation 7: 617-630.

Bongers A. M. T. 1988. De nematoden van Nederland. - Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging.

Bonte D. and Maelfait J. P. 1998. Levenscycli van enkele duinspinnen/life cycle reconstruction of coastal dune spiders. - Nieuwsbrief van de Belgische Arachnologische Vereniging/Newsletter of the Belgian Arachnological Society 13: 1-15.

Bonte D., Maelfait J. P. and Hoffmann M. 2000. Seasonal and diurnal migration patterns of the spider (Araneae) fauna of coastal grey dunes. - Ekologia-Bratislava 19: 5-16.

Bonte D. and Maelfait J. P. 2001. Life history, habitat use and dispersal of a dune wolf spider (*Pardosa monticola* (clerck, 1757) Lycosidae, Araneae) in the Flemish coastal dunes (Belgium). - Belgian Journal of Zoology 131: 145-157.

Bonte D., Lens L. and Maelfait J. P. 2006. Sand dynamics in coastal dune landscapes constrain diversity and life-history characteristics of spiders. - Journal of Applied Ecology 43: 735-747.

Bonte D., De Roissart A., Vandegehuchte M. L., Ballhorn D. J., Van Leeuwen T. and de la Peña E. 2010. Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota. - PLoS ONE 5: e11174.

Borowicz V. A. 1997. A fungal root symbiont modifies plant resistance to an insect herbivore. - Oecologia 112: 534-542.

Boudreau S. and Houle G. 2001. The *Ammophila* decline: A field experiment on the effects of mineral nutrition. - Ecoscience 8: 392-398.

Brinkman E. P., van Veen J. A. and van der Putten W. H. 2004. Endoparasitic nematodes reduce multiplication of ectoparasitic nematodes, but do not prevent growth reduction of *Ammophila arenaria* (L.) Link (marram grass). - Applied Soil Ecology 27: 65-75.

Brinkman E. P., Duyts H. and Van Der Putten W. H. 2005a. Consequences of variation in species diversity in a community of root-feeding herbivores for nematode dynamics and host plant biomass. - Oikos 110: 417-427.

Brinkman E. P., Duyts H. and Van Der Putten W. H. 2005b. Competition between endoparasitic nematodes and effect on biomass of *Ammophila arenaria* (marram grass) as affected by timing of inoculation and plant age. - Nematology 7: 169-178.

Brinkman E. P., Troelstra S. R. and van der Putten W. H. 2005c. Soil feedback effects to the foredune grass *Ammophila arenaria* by endoparasitic root-feeding nematodes and whole soil communities. - Soil Biology & Biochemistry 37: 2077-2087.

Brundrett M. 1991. Mycorrhizas in natural ecosystems. - Advances in Ecological Research 21: 171-313.

Brussaard L., Pulleman M. M., Ouedraogo E., Mando A. and Six J. 2007. Soil fauna and soil function in the fabric of the food web. - Pedobiologia 50: 447-462.

Brzeski M. W. 1998. Nematodes of Tylenchina in Poland and temperate Europe. - Muzeum i Instytut Zoologii Polska Akademia Nauk.

Buchenau F. 1888. Über die Vegetationsverhältnisse des 'Helms' (*Psamma arenaria* Röm. & Schultes) und der verwandten Dünengräser. - Naturwissenschaftlicher Verein zu Bremen 10: 397-412.

Chun Y. J., van Kleunen M. and Dawson W. 2010. The role of enemy release, tolerance and resistance in plant invasions: Linking damage to performance. - Ecology Letters 13: 937-946.

Cohen J. E., Pimm S. L., Yodzis P. and Saldana J. 1993. Body sizes of animal predators and animal prey in food webs. - Journal of Animal Ecology 62: 67-78.

Coley P. D., Bateman M. L. and Kursar T. A. 2006. The effects of plant quality on caterpillar growth and defense against natural enemies. - Oikos 115: 219-228.

Crutsinger G. M., Reynolds W. N., Classen A. T. and Sanders N. J. 2008. Disparate effects of plant genotypic diversity on foliage and litter arthropod communities. - Oecologia 158: 65-75.

Dawkins R. 1982. The extended phenotype. - Oxford University Press.

Day F. P., Conn C., Crawford E. and Stevenson M. 2004. Long-term effects of nitrogen fertilization on plant community structure on a coastal barrier island dune chronosequence. - Journal of Coastal Research 20: 722-730.

De Deyn G. B., Van Ruijven J., Raaijmakers C. E., De Ruiter P. C. and Van Der Putten W. H. 2007. Above- and belowground insect herbivores differentially affect soil nematode communities in species-rich plant communities. - Oikos 116: 923-930.

de la Peña E., Rodríguez-Echeverría S., van der Putten W. H., Freitas H. and Moens M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. - New Phytologist 169: 829-840.

de la Peña E., Karssen G. and Moens M. 2007. Distribution and diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with *Ammophila arenaria* in coastal dunes of Western Europe. - Nematology 9: 881-901.

de la Peña E., Vandegehuchte M., Bonte D. and Moens M. 2008. Analysis of the specificity of three root-feeders towards grasses in coastal dunes. - Plant and Soil 310: 113-120.

de la Peña E., Bonte D. and Moens M. 2009. Evidence of population differentiation in the dune grass *Ammophila arenaria* and its associated root-feeding nematodes. - Plant and Soil 324: 307-316.

de la Peña E., D'hondt B. and Bonte D. 2010. Landscape structure, dispersal and the evolution of antagonistic plant-herbivore interactions. - Ecography in press.

De Rooij - Van der Goes P. C. E. M. 1995. The role of plant-parasitic nematodes and soil-borne fungi in the decline of *Ammophila arenaria* (L) Link. - New Phytologist 129: 661-669.

DeWalt S. J., Denslow J. S. and Ickes K. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. - Ecology 85: 471-483.

Dhileepan K. 2004. The applicability of the plant vigor and resource regulation hypotheses in explaining *Epiblema* gall moth-*Parthenium* weed interactions. - Entomologia Experimentalis et Applicata 113: 63-70.

Eldred R. A. and Maun M. A. 1982. A multivariate approach to the problem of decline in vigor of *Ammophila*. - Canadian Journal of Botany-Revue Canadienne De Botanique 60: 1371-1380.

Falconer D. S. and Mackay T. F. C. 1996. Introduction to quantitative genetics. - Longman Group Ltd.

Fant J. B., Holmstrom R. M., Sirkin E., Etterson J. R. and Masi S. 2008. Genetic structure of threatened native populations and propagules used for restoration in a clonal species, american beachgrass (*Ammophila breviligulata* Fern.). - Restoration Ecology 16: 594-603.

Fitter A. H. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. - New Phytologist 172: 3-6.

Fontana A., Reichelt M., Hempel S., Gershenzon J. and Unsicker S. B. 2009. The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of *Plantago lanceolata* L. - Journal of Chemical Ecology 35: 833-843.

Freude H., Harde K. W. and Lohse G. A. 1981. Die Käfer Mitteleuropas. Band 10. Bruchidae - Curculionidae I. - Goecke & Evers.

Fritz R. S. and Price P. W. 1988. Genetic variation among plants and insect community structure - willows and sawflies. - Ecology 69: 845-856.

Fritz R. S. 1990. Effects of genetic and environmental variation on resistance of willow to sawflies. - Oecologia 82: 325-332.

Fuller V. L., Lilley C. J. and Urwin P. E. 2008. Nematode resistance. - New Phytologist 180: 27-44.

Futuyma D. J. 2005. Evolution. - Sinauer Associates, inc.

Gange A. C. and Brown V. K. 1989. Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. - Oecologia 81: 38-42.

Gange A. C. and Nice H. E. 1997. Performance of the thistle gall fly, *Urophora cardui*, in relation to host plant nitrogen and mycorrhizal colonization. - New Phytologist 137: 335-343.

Gange A. C. and Ayres R. L. 1999. On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. - Oikos 87: 615-621.

Gange A. C., Bower E. and Brown V. K. 1999. Positive effects of an arbuscular mycorrhizal fungus on aphid life history traits. - Oecologia 120: 123-131.

Gange A. C., Brown V. K. and Aplin D. M. 2005. Ecological specificity of arbuscular mycorrhizae: Evidence from foliar- and seed-feeding insects. - Ecology 86: 603-611.

Gittenberger E., Backhuys W. and Ripken T. E. J. 1984. De landslakken van Nederland. - KNNV.

González-Megías A. G. and Müller C. 2010. Root herbivores and detritivores shape above-ground multitrophic assemblage through plant-mediated effects. - Journal of Animal Ecology 79: 923-931.

Goverde M., van der Heijden M. G. A., Wiemken A., Sanders I. R. and Erhardt A. 2000. Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. - Oecologia 125: 362-369.

Graham J. H. and Abbott L. K. 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. - Plant and Soil 220: 207-218.

Green D. L. 1965. Developmental history of European beachgrass (*Ammophila arenaria* (L.) Link) plantings on the Oregon coastal sand dunes. - Oregon State University, p. 64.

Guerrieri E., Lingua G., Digilio M. C., Massa N. and Berta G. 2004. Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? - Ecological Entomology 29: 753-756.

Haase J., Brandl R., Scheu S. and Schadler M. 2008. Above- and belowground interactions are mediated by nutrient availability. - Ecology 89: 3072-3081.

Halwagy R. 1953. On the growth and relative distribution of *Psamma arenaria* Roem. et Schult, and *Carex arenaria* L. on the sand dunes at Blakeney Point, Norfolk. - University of London.

Harrison M. J. 2005. Signaling in the arbuscular mycorrhizal symbiosis. - Annual Review of Microbiology 59: 19-42.

Hartley S. E. and Gange A. C. 2009. Impacts of plant symbiotic fungi on insect herbivores: Mutualism in a multitrophic context. - Annual Review of Entomology 54: 323-342.

Hassouna M. G. and Wareing P. F. 1964. Possible role of rhizosphere bacteria in nitrogen nutrition of *Ammophila arenaria*. - Nature 202: 467-469.

Heie O. E. 1980. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I. General part. The families Mindaridae, Hormaphidae, Thelaxidae, Anoeciidae, and Pemphigidae. Fauna Entomologica Scandinavica, vol. 9. - Scandinavian Science Press Ltd.

Heie O. E. 1982. The Aphidoidea (Hemiptera) of Fennoscandia and Fenmark. II. The family Drepanosiphidae. Fauna Entomologica Scandinavica, vol. 11. - Scandinavian Science Press Ltd.

Heie O. E. 1986. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III. Family Aphididae: Subfamily Pterocommatinae & tribe Aphidini of subfamily Aphidinae. Fauna Entomologica Scandinavica, vol. 17. - E.J. Brill/Scandinavian Science Press Ltd.

Heie O. E. 1992. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. IV. Family Aphididae: Part 1 of tribe Macrosiphini of subfamily Aphidinae. Fauna Entomologica Scandinavica, vol. 25. - E.J. Brill/Scandinavian Science Press Ltd.

Heie O. E. 1994. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. V. Family Aphididae: Part 2 of tribe Macrosiphini of subfamily Aphidinae. Fauna Entomologica Scandinavica, vol. 28. - E.J. Brill.

Heie O. E. 1995. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. VI. Family Aphididae: Part 3 of tribe Macrosiphini of subfamily Aphidinae, and family Lachnidae. Fauna Entomologica Scandinavica, vol. 31. - E.J. Brill.

Hendrickx G. 1995. An automatic apparatus for extracting free-living nematode stages from soil. - Nematologica 41: 308.

Hertling U. M. and Lubke R. A. 2000. Assessing the potential for biological invasion - the case of *Ammophila arenaria* in South Africa. - South African Journal of Science 96: 520-527.

Heyligers P. C. 1985. The impact of introduced plants on foredune formation in South Eastern Australia. - Proceedings of the Ecological Society of Australia 14: 23-41.

Hille Ris Lambers D. 1950. On mounting aphids and other soft-skinned insects. - Entomologische Berichten (Amsterdam) 298: 55-58.

Hilton M., Duncan M. and Jul A. 2005. Processes of *Ammophila arenaria* (marram grass) invasion and indigenous species displacement, Stewart Island, New Zealand. - Journal of Coastal Research 21: 175-185.

Hodge A. 2000. Microbial ecology of the arbuscular mycorrhiza. - FEMS Microbiology Ecology 32: 91-96.

Hoeksema J. D., Chaudhary V. B., Gehring C. A., Johnson N. C., Karst J., Koide R. T., Pringle A., Zabinski C., Bever J. D., Moore J. C., Wilson G. W. T., Klironomos J. N. and Umbanhowar J. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. - Ecology Letters 13: 394-407.

Hoffmann D., Vierheilig H., Riegler P. and Schausberger P. 2009. Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite *Tetranychus urticae*. - Oecologia 158: 663-671.

Hol W. H. G., de la Pena E., Moens M. and Cook R. 2007. Interaction between a fungal endophyte and root herbivores of *Ammophila arenaria*. - Basic and Applied Ecology 8: 500-509.

Hol W. H. G., van der Wurff A. W. G., Skøt L. and Cook R. 2008. Two distinct AFLP types in three populations of marram grass (*Ammophila arenaria*) in Wales. - Plant Genetic Resources Characterization and Utilization 6: 201-207.

Holman J. 2009. Host plant catalog of aphids: Palearctic region. - Springer Verlag.

Holzinger W. E., Kammerlander I. and Nickel H. 2003. The Auchenorrhyncha of Central Europe. Volume 1: Fulgoromorpha, Cicadomorpha excl. Cicadellidae. - Brill.

Hougen-Eitzman D. and Rausher M. D. 1994. Interactions between herbivorous insects and plant-insect coevolution. - American Naturalist 143: 677-697.

Huberty A. F. and Denno R. F. 2004. Plant water stress and its consequences for herbivorous insects: A new synthesis. - Ecology 85: 1383-1398.

Huberty A. F. and Denno R. F. 2006. Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. - Oecologia 149: 444-455.

Huiskes A. H. L. 1977. Population dynamics of *Ammophila arenaria* (L.) Link. - University of Wales.

Huiskes A. H. L. 1979. Biological flora of the British Isles – *Ammophila arenaria* (L) Link (*Psamma arenaria* (L) Roem et Schult – *Calamagrostis arenaria* (L) Roth). - Journal of Ecology 67: 363-382.

Inouye B. and Stinchcombe J. R. 2001. Relationships between ecological interaction modifications and diffuse coevolution: Similarities, differences, and causal links. - Oikos 95: 353-360.

Iwao K. and Rausher M. D. 1997. Evolution of plant resistance to multiple herbivores: Quantifying diffuse coevolution. - American Naturalist 149: 316-335.

Janzen D. H. 1980. When is it coevolution? - Evolution 34: 611-612.

Johnson M. T. J., Lajeunesse M. J. and Agrawal A. A. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. - Ecology Letters 9: 24-34.

Johnson M. T. J. and Agrawal A. A. 2007. Covariation and composition of arthropod species across plant genotypes of evening primrose (*Oenothera biennis*). - Oikos 116: 941-956.

Johnson N. C., Graham J. H. and Smith F. A. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. - New Phytologist 135: 575-586.

Johnson S. N., Hawes C. and Karley A. J. 2009. Reappraising the role of plant nutrients as mediators of interactions between root- and foliar-feeding insects. - Functional Ecology 23: 699-706.

Joosten L., Mulder P. P. J., Klinkhamer P. G. L. and van Veen J. A. 2009. Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in J*acobaea vulgaris*. - Plant and Soil 325: 133-143.

Kaplan I., Halitschke R., Kessler A., Rehill B. J., Sardanelli S. and Denno R. F. 2008. Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory. - Ecology Letters 11: 841-851.

Kaplan I., Sardanelli S. and Denno R. F. 2009. Field evidence for indirect interactions between foliar-feeding insect and root-feeding nematode communities on *Nicotiana tabacum*. - Ecological Entomology 34: 262-270.

Karssen G., van Aelst A. and van der Putten W. H. 1998. *Meloidogyne duytsi* n. sp. (Nematoda : Heteroderidae), a root-knot nematode from Dutch coastal foredunes. - Fundamental and Applied Nematology 21: 299-306.

Karssen G. and Moens M. 2006. Root-knot nematodes. - In: Plant nematology. CABI Publishing, pp. 59-90.

Keane R. M. and Crawley M. J. 2002. Exotic plant invasions and the enemy release hypothesis. - Trends in Ecology & Evolution 17: 164-170.

Kempel A., Schmidt A. K., Brandl R. and Schadler M. 2010. Support from the underground: Induced plant resistance depends on arbuscular mycorrhizal fungi. - Functional Ecology 24: 293-300.

Khan M. and Port G. 2008. Performance of clones and morphs of two cereal aphids on wheat plants with high and low nitrogen content. - Entomological Science 11: 159-165.

Klironomos J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. - Ecology 84: 2292-2301.

Knevel I. C., Lans T., Menting F. B. J., Hertling U. M. and van der Putten W. H. 2004. Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. - Oecologia 141: 502-510.

Koide R. 1985. The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. - New Phytologist 99: 449-462.

Kooijman A. M. and Besse M. 2002. The higher availability of N and P in lime-poor than in lime-rich coastal dunes in the Netherlands. - Journal of Ecology 90: 394-403.

Koricheva J., Gange A. C. and Jones T. 2009. Effects of mycorrhizal fungi on insect herbivores: A meta-analysis. - Ecology 90: 2088-2097.

Kowalchuk G. A., De Souza F. A. and Van Veen J. A. 2002. Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch coastal sand dunes. - Molecular Ecology 11: 571-581.

Leimu R. and Koricheva J. 2006. A meta-analysis of genetic correlations between plant resistances to multiple enemies. - American Naturalist 168: E15-E37.

Little L. R. and Maun M. A. 1996. The '*Ammophila* problem' revisited: A role for mycorrhizal fungi. - Journal of Ecology 84: 1-7.

Liu H. and Stiling P. 2006. Testing the enemy release hypothesis: A review and meta-analysis. - Biological Invasions 8: 1535-1545.

Lynch M. and Milligan B. G. 1994. Analysis of population genetic structure with RAPD markers. - Molecular Ecology 3: 91-99.

Maddox G. D. and Root R. B. 1990. Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. - Ecology 71: 2115-2124.

Maron J. L. and Vila M. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. - Oikos 95: 361-373.

Marshall J. K. 1965. *Corynephorus canescens* (L) P Beauv as a model for the *Ammophila* problem. - Journal of Ecology 53: 447-&.

Massart J. 1912. La cinquantième herborisation générale de la Société Royale de Botanique de Belgique sur le littoral Belge. - Bulletin de la Société Royale de Botanique de Belgique 51: 1-185.

Masters G. J., Brown V. K. and Gange A. C. 1993. Plant mediated interactions between aboveground and belowground insect herbivores. - Oikos 66: 148-151.

Masters G. J. 1995. The impact of root herbivory on aphid performance - field and laboratory evidence. - Acta Oecologica-International Journal of Ecology 16: 135-142.

Masters G. J., Jones T. H. and Rogers M. 2001. Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. - Oecologia 127: 246-250.

McCune B. and Grace J. B. 2002. Analysis of ecological communities. - MjM Software Design.

McGuinness K. A. 2002. Of rowing boats, ocean liners and tests of the ANOVA homogeneity of variance assumption. - Austral Ecology 27: 681-688.

Memmott J., Martinez N. D. and Cohen J. E. 2000. Predators, parasitoids and pathogens: Species richness, trophic generality and body sizes in a natural food web. - Journal of Animal Ecology 69: 1-15.

Memmott J. 2009. Food webs: A ladder for picking strawberries or a practical tool for practical problems? - Philosophical Transactions of the Royal Society B-Biological Sciences 364: 1693-1699.

Moran N. A. and Whitham T. G. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. - Ecology 71: 1050-1058.

Nentwig W. 1987. Feeding ecology. - In: Ecophysiology of spiders. Springer-Verlag, pp. 249-263.

Neter J., Kutner M. H., Nachtsheim C. J. and Wasserman W. 1996. Applied linear statistical models. - WCB/McGraw-Hill.

Neveu N., Grandgirard J., Nenon J. P. and Cortesero A. M. 2002. Systemic release of herbivore-induced plant volatiles by turnips infested by concealed root-feeding larvae *Delia radicum* L. - Journal of Chemical Ecology 28: 1717-1732.

Newton E. L., Bullock J. M. and Hodgson D. J. 2009. Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. - Oecologia 160: 63-76.

Nickel H. 2003. The leafhoppers and planthoppers of germany (Hemiptera, Auchenorrhyncha): Patterns and strategies in a highly diverse group of phytophagous insects. - Pensoft Publishers, Sofia-Moscow and Goecke & Evers, Keltern.

Nicolson T. H. 1960. Mycorrhiza in the Gramineae: II. Development in different habitats, particularly sand dunes. - Transactions of the British Mycological Society 43: 132-145.

Nicolson T. H. 1979. Mycorrhiza in the gramineae: III. *Glomus fasciculatus* as the endophyte of pioneer grasses in a maritime sand dune. - Transactions of the British Mycological Society 72: 261-268.

Olsen S. R., Cole C. V., Watanabe F. S. and Dean L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. - US Department of Agriculture Circular 939: 1-19.

Orians C. M. and Fritz R. S. 1996. Genetic and soil-nutrient effects on the abundance of herbivores on willow. - Oecologia 105: 388-396.

Park M. G. and Blossey B. 2008. Importance of plant traits and herbivory for invasiveness of *Phragmites australis* (Poaceae). - American Journal of Botany 95: 1557-1568.

Peakall R. and Smouse P. E. 2006. Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. - Molecular Ecology Notes 6: 288-295.

Peter C. I. 2000. Water requirements and distribution of *Ammophila arenaria* and *Scaevola plumieri* on South African coastal dunes. Department of Botany. - Rhodes University, p. 167.

Petit R. J. 2004. Biological invasions at the gene level. - Diversity and Distributions 10: 159-165.

Pickart A. J. 1997. Control of European beachgrass (*Ammophila arenaria*) on the west coast of the United States. - Proceedings of the California Exotic Pest Plant Council Symposium 1997, pp. 82-90.

Pilson D. 2000. The evolution of plant response to herbivory: Simultaneously considering resistance and tolerance in *Brassica rapa*. - Evolutionary Ecology 14: 457-489.

Pimm S. L., Lawton J. H. and Cohen J. E. 1991. Food web patterns and their consequences. - Nature 350: 669-674.

Piskiewicz A. M., Duyts H., Berg M. P., Costa S. R. and van der Putten W. H. 2007. Soil microorganisms control plant ectoparasitic nematodes in natural coastal foredunes. - Oecologia 152: 505-514.

Piskiewicz A. M., Duyts H. and van der Putten W. H. 2008. Multiple species-specific controls of root-feeding nematodes in natural soils. - Soil Biology & Biochemistry 40: 2729-2735.

Piskiewicz A. M., Duyts H. and van der Putten W. H. 2009. Soil microorganisms in coastal foredunes control the ectoparasitic root-feeding nematode *Tylenchorhynchus ventralis* by local interactions. - Functional Ecology 23: 621-626.

Poll J., Marhan S., Haase S., Hallmann J., Kandeler E. and Ruess L. 2007. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. - FEMS Microbiology Ecology 62: 268-279.

Poveda K., Steffan-Dewenter I., Scheu S. and Tscharntke T. 2005. Effects of decomposers and herbivores on plant performance and aboveground plant-insect interactions. - Oikos 108: 503-510.

Price P. W. 1991. The plant vigor hypothesis and herbivore attack. - Oikos 62: 244-251.

Provoost S. and Van Landuyt W. 2001. The flora of the Flemish coastal dunes (Belgium) in a changing landscape. - In: Houston, J. A., Edmondson, S. E. and Rooney, P. J. (eds.), Coastal dune management, shared experience of European conservation practice. Liverpool University Press, pp. 393-401.

Provoost S., Jones M. L. M. and Edmondson S. E. 2010. Changes in landscape and vegetation of coastal dunes in northwest Europe: A review. - Journal of Coastal Conservation. in press.

Reynolds H. L., Vogelsang K. M., Hartley A. E., Bever J. D. and Schultz P. A. 2006. Variable responses of old-field perennials to arbuscular mycorrhizal fungi and phosphorus source. - Oecologia 147: 348-358.

Reynolds O. L., Keeping M. G. and Meyer J. H. 2009. Silicon-augmented resistance of plants to herbivorous insects: A review. - Annals of Applied Biology 155: 171-186.

Ribaut H. 1952. Homoptères Auchenorhynques II (Jassidae). - Faune de France 57: 1-474.

Rice W. R. and Gaines S. D. 1994a. Extending nondirectional heterogeneity tests to evaluate simply ordered alternative hypotheses. - Proceedings of the National Academy of Sciences of the United States of America 91: 225-226.

Rice W. R. and Gaines S. D. 1994b. The ordered-heterogeneity family of tests. - Biometrics 50: 746-752.

Richardson D. M. and Pysek P. 2008. Fifty years of invasion ecology - the legacy of Charles Elton. - Diversity and Distributions 14: 161-168.

Roche B. M. and Fritz R. S. 1997. Genetics of resistance of *Salix sericea* to a diverse community of herbivores. - Evolution 51: 1490-1498.

Rodríguez-Echeverría S., Freitas H. and van der Putten W. H. 2008a. Genetic diversity and differentiation *of Ammophila arenaria* (L.) Link as revealed by ISSR markers. - Journal of Coastal Research 24: 122-126.

Rodríguez-Echeverría S., Hol W. H. G., Freitas H., Eason W. R. and Cook R. 2008b. Arbuscular mycorrhizal fungi of *Ammophila arenaria* (L.) Link: Spore abundance and root colonisation in six locations of the European coast. - European Journal of Soil Biology 44: 30-36.

Rowntree J. K., McVennon A. and Preziosi R. F. 2010. Plant genotype mediates the effects of nutrients on aphids. - Oecologia in press.

Salt D. T., Fenwick P. and Whittaker J. B. 1996. Interspecific herbivore interactions in a high CO_2 environment: Root and shoot aphids feeding on *Cardamine*. - Oikos 77: 326-330.

Saltonstall K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. - Proceedings of the National Academy of Sciences of the United States of America 99: 2445-2449.

Sanchez-Moreiras A. M., Weiss O. A. and Reigosa-Roger M. J. 2003. Allelopathic evidence in the Poaceae. - Botanical Review 69: 300-319.

Sauer D. B. and Burroughs R. 1986. Disinfection of seed surfaces with sodium hypochlorite. - Phytopathology 76: 745-749.

Scheu S. 2001. Plants and generalist predators as links between the below-ground and above-ground system. - Basic and Applied Ecology 2: 3-13.

Schierenbeck K. A., Mack R. N. and Sharitz R. R. 1994. Effects of herbivory on growth and biomass allocation in native and introduced species of *Lonicera*. - Ecology 75: 1661-1672.

Schoonhoven L. M., van Loon J. J. A. and Dicke M. 2005. Insect-plant biology. - Oxford University Press.

Schreck Reis C., Freitas H. and van der Putten W. H. 2008. Responses of root-feeding nematodes (*Helicotylenchus* spp.) to local and non-local, populations of the host plant *Ammophila arenaria*. - Applied Soil Ecology 39: 245-253.

Schweitzer J. A., Bailey J. K., Fischer D. G., Leroy C. J., Lonsdorf E. V., Whitham T. G. and Hart S. C. 2008. Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. - Ecology 89: 773-781.

Seastedt T. R., Ramundo R. A. and Hayes D. C. 1988. Maximization of densities of soil animals by foliage herbivory - empirical evidence, graphical and conceptual models. - Oikos 51: 243-248.

Seastedt T. R. and Reddy M. V. 1991. Fire, mowing and insecticide effects on soil Sternorrhyncha (Homoptera) densities in tallgrass prairie. - Journal of the Kansas Entomological Society 64: 238-242.

Seliskar D. M. and Huettel R. N. 1993. Nematode involvement in the dieout of *Ammophila breviligulata* (Poaceae) on the mid-Atlantic coastal dunes of the United States. - Journal of Coastal Research 9: 97-103.

Shen C. S. and Bach C. E. 1997. Genetic variation in resistance and tolerance to insect herbivory in *Salix cordata*. - Ecological Entomology 22: 335-342.

Slobodchikoff C. N. and Doyen J. T. 1977. Effects of *Ammophila arenaria* on sand dune arthropod communities. - Ecology 58: 1171-1175.

Smiley R. W., Whittaker R. G., Gourlie J. A. and Easley S. A. 2005. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. - Plant Disease 89: 958-968.

Smith S. E. and Read D. J. 1997. Mycorrhizal symbiosis. - Academic Press.

Soler R., Bezemer T. M., Van Der Putten W. H., Vet L. E. M. and Harvey J. A. 2005. Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. - Journal of Animal Ecology 74: 1121-1130.

Soler R., Bezemer T. M., Cortesero A. M., Van Der Putten W. H., Vet L. E. M. and Harvey J. A. 2007a. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. - Oecologia 152: 257-264.

Soler R., Harvey J. A. and Bezemer T. M. 2007b. Foraging efficiency of a parasitoid of a leaf herbivore is influenced by root herbivory on neighbouring plants. - Functional Ecology 21: 969-974.

Soler R., Harvey J. A., Kamp A. F. D., Vet L. E. M., Van der Putten W. H., Van Dam N. M., Stuefer J. F., Gols R., Hordijk C. A. and Bezemer T. M. 2007c. Root herbivores influence the behaviour of an aboveground parasitoid through changes in plant-volatile signals. - Oikos 116: 367-376.

Staley J. T., Mortimer S. R., Morecroft M. D., Brown V. K. and Masters G. J. 2007. Summer drought alters plant-mediated competition between foliar- and root-feeding insects. - Global Change Biology 13: 866-877.

Staley J. T., Mortimer S. R. and Morecroft M. D. 2008. Drought impacts on above-belowground interactions: Do effects differ between annual and perennial host species? - Basic and Applied Ecology 9: 673-681.

Steane D. A., Conod N., Jones R. C., Vaillancourt R. E. and Potts B. M. 2006. A comparative analysis of population structure of a forest tree, *Eucalyptus globutus* (Myrtaceae), using microsatellite markers and quantitative traits. - Tree Genetics & Genomes 2: 30-38.

Sternberg M. 2000. Terrestrial gastropods and experimental climate change: A field study in a calcareous grassland. - Ecological Research 15: 73-81.

Stinchcombe J. R. and Rausher M. D. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. - American Naturalist 158: 376-388.

Strauss S. Y., Rudgers J. A., Lau J. A. and Irwin R. E. 2002. Direct and ecological costs of resistance to herbivory. - Trends in Ecology & Evolution 17: 278-285.

Sudova R. and Vosatka M. 2008. Effects of inoculation with native arbuscular mycorrhizal fungi on clonal growth of *Potentilla reptans* and *Fragaria moschata* (Rosaceae). - Plant and Soil 308: 55-67.

Sutton S. L. 1972. Woodlice. - Ginn & Company Limited.

Tikkanen O. P., Rousi M., Ylioja T. and Roininen H. 2003. No negative correlation between growth and resistance to multiple herbivory in a deciduous tree, *Betula pendula*. - Forest Ecology and Management 177: 587-592.

Tindall K. V. and Stout M. J. 2001. Plant-mediated interactions between the rice water weevil and fall armyworm in rice. - Entomologia Experimentalis et Applicata 101: 9-17.

Toju H. 2009. Natural selection drives the fine-scale divergence of a coevolutionary arms race involving a long-mouthed weevil and its obligate host plant. - BMC Evolutionary Biology 9: 273.

Travers-Martin N. and Müller C. 2008. Matching plant defence syndromes with performance and preference of a specialist herbivore. - Functional Ecology 22: 1033-1043.

Tsoar H. 2005. Sand dunes mobility and stability in relation to climate. - Physica a-Statistical Mechanics and Its Applications 357: 50-56.

Tuomisto H. 2010a. A diversity of beta diversities: Straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. - Ecography 33: 2-22.

Tuomisto H. 2010b. A diversity of beta diversities: Straightening up a concept gone awry. Part 2. Quantifying beta diversity and related phenomena. - Ecography 33: 23-45.

van Dam N. M., Harvey J. A., Wackers F. L., Bezemer T. M., van der Putten W. H. and Vet L. E. M. 2003. Interactions between aboveground and belowground induced responses against phytophages. - Basic and Applied Ecology 4: 63-77.

van der Putten W. H., Vandijk C. and Troelstra S. R. 1988. Biotic soil factors affecting the growth and development of *Ammophila arenaria*. - Oecologia 76: 313-320.

van der Putten W. H. 1990. Establishment of *Ammophila arenaria* (marram grass) from culms, seeds and rhizomes. - Journal of Applied Ecology 27: 188-199.

van der Putten W. H., Maas P. W. T., Van Gulik W. J. M. and Brinkman H. 1990. Characterization of soil organisms involved in the degeneration of *Ammophila arenaria*. - Soil Biology & Biochemistry 22: 845-852.

van der Putten W. H. and Kloosterman E. H. 1991. Large-scale establishment of *Ammophila arenaria* and quantitative assessment by remote-sensing. - Journal of Coastal Research 7: 1181-1194.

van der Putten W. H., Vandijk C. and Peters B. A. M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. - Nature 362: 53-56.

van der Putten W. H. and van der Stoel C. D. 1998. Plant parasitic nematodes and spatio-temporal variation in natural vegetation. - Applied Soil Ecology 10: 253-262.

van der Putten W. H., Vet L. E. M., Harvey J. A. and Wackers F. L. 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. - Trends in Ecology & Evolution 16: 547-554.

van der Putten W. H., Yeates G. W., Duyts H., Schreck Reis C. and Karssen G. 2005. Invasive plants and their escape from root herbivory: A worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. - Biological Invasions 7: 733-746.

van der Putten W. H., Cook R., Costa S., Davies K. G., Fargette M., Freitas H., Hol W. H. G., Kerry B. R., Maher N., Mateille T., Moens M., de la Peña E., Piskiewicz A. M., Raeymaekers A. D. W., Rodríguez-Echeverría S. and van der Wurff A. W. G. 2006. Nematode interactions in nature: Models for sustainable control of nematode pests of crop plants? Advances in Agronomy 89: 227-260.

van der Putten W. H., Bardgett R. D., de Ruiter P. C., Hol W. H. G., Meyer K. M., Bezemer T. M., Bradford M. A., Christensen S., Eppinga M. B., Fukami T., Hemerik L., Molofsky J., Schadler M., Scherber C., Strauss S. Y., Vos M. and Wardle D. A. 2009. Empirical and theoretical challenges in aboveground-belowground ecology. - Oecologia 161: 1-14.

van der Stoel C. D., van der Putten W. H. and Duyts H. 2002. Development of a negative plant-soil feedback in the expansion zone of the clonal grass *Ammophila arenaria* following root formation and nematode colonization. - Journal of Ecology 90: 978-988.

van der Stoel C. D., Duyts H. and van der Putten W. H. 2006. Population dynamics of a host-specific root-feeding cyst nematode and resource quantity in the root zone of a clonal grass. - Oikos 112: 651-659.

van der Stoel C. D. and van der Putten W. H. 2006. Pathogenicity and host range of *Heterodera arenaria* in coastal foredunes. - Nematology 8: 255-263.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010a. Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. - Oikos 119: 1011-1019.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010b. Contrasting covariation of above- and belowground invertebrate species across plant genotypes. - Journal of Animal Ecology in press.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010c. Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics. - PLoS ONE 5: e11174.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010d. Aphids on *Ammophila arenaria* in Belgium: First reports, phenology and host range expansion. - Belgian Journal of Zoology 140: 77-79.

Vanden Borre J., Bonte D. and Maelfait J. P. 2006. Interdemic variation of cannibalism in a wolf spider (*Pardosa monticola*) inhabiting different habitat types. - Ecological Entomology 31: 99-105.

Vandenbossche B. Ecological linkages between above- and belowground herbivores: Insights from the interaction between plant-parasitic nematodes and aphids in the dune grass *Ammophila arenaria*. - Ghent University, p. 29.

Vander Mijnsbrugge K., Bischoff A. and Smith B. 2010. A question of origin: Where and how to collect seed for ecological restoration. - Basic and Applied Ecology 11: 300-311.

Voesenek L., van der Putten W. H., Maun M. A. and Blom C. 1998. The role of ethylene and darkness in accelerated shoot elongation of *Ammophila breviligulata* upon sand burial. - Oecologia 115: 359-365.

Vos P., Hogers R., Bleeker M., Reijans M., Vandelee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. 1995. AFLP - a new technique for DNA-fingerprinting. - Nucleic Acids Research 23: 4407-4414.

Wall J. W., Skene K. R. and Neilson R. 2002. Nematode community and trophic structure along a sand dune succession. - Biology and Fertility of Soils 35: 293-301.

Wallén B. 1980. Changes in structure and function of *Ammophila* during primary succession. - Oikos 34: 227-238.

Wardle D. A. 2002. Communities and ecosystems: Linking the aboveground and belowground components. - Princeton University Press.

Wardle D. A., Bardgett R. D., Klironomos J. N., Setala H., Van Der Putten W. H. and Wall D. H. 2004. Ecological linkages between aboveground and belowground biota. - Science 304: 1629-1633.

Webb C. E., Oliver I. and Pik A. J. 2000. Does coastal foredune stabilization with *Ammophila arenaria* restore plant and arthropod communities in southeastern Australia? - Restoration Ecology 8: 283-288.

Weeda E. J., Westra R., Westra C. and Westra T. 1994. Nederlandse oecologische flora: Wilde planten en hun relaties. - IVN.

Welch B. L. 1951. On the comparison of several mean values: An alternative approach. - Biometrika 38: 330–336.

White T. C. R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. - Ecology 50: 905-909.

White T. C. R. 1978. Importance of a relative shortage of food in animal ecology. - Oecologia 33: 71-86.

White T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. - Oecologia 63: 90-105.

White T. C. R. 2009. Plant vigour versus plant stress: A false dichotomy. - Oikos 118: 807-808.

Whitehead A. G. and Hemming J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. - Annals of Applied Biology 55: 25-38.

Whitfield C. J. and Brown R. L. 1948. Grasses that fix sand dunes. - In: U. S. Department of agriculture. Yearbook., pp. 70-74.

Whitham T. G., Young W. P., Martinsen G. D., Gehring C. A., Schweitzer J. A., Shuster S. M., Wimp G. M., Fischer D. G., Bailey J. K., Lindroth R. L., Woolbright S. and Kuske C. R. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. - Ecology 84: 559-573.

Whitham T. G., Bailey J. K., Schweitzer J. A., Shuster S. M., Bangert R. K., Leroy C. J., Lonsdorf E. V., Allan G. J., DiFazio S. P., Potts B. M., Fischer D. G., Gehring C. A., Lindroth R. L., Marks J. C., Hart S. C., Wimp G. M. and Wooley S. C. 2006. A framework for community and ecosystem genetics: From genes to ecosystems. - Nature Reviews Genetics 7: 510-523.

Wiedemann A. M. and Pickart A. 1996. The *Ammophila* problem on the northwest coast of North America. - Landscape and Urban Planning 34: 287-299.

Wijnhoven H. 2009. De Nederlandse hooiwagens (Opiliones). - Entomologische Tabellen 3: 1-118.

Willis A. J., Folkes B. F., Hope-Simpson J. F. and Yemm E. W. 1959a. Braunton Burrows: The dune system and its vegetation. 1. - Journal of Ecology: 47: 1-24.

Willis A. J., Folkes B. F., Hope-Simpson J. F. and Yemm E. W. 1959b. Braunton Burrows: The dune system and its vegetation. 2. - Journal of Ecology: 47: 249-288.

Willis A. J. 1965. The influence of mineral nutrients on the growth of *Ammophila arenaria*. - Journal of Ecology 53: 735-745.

Wise D. H. 1995. Spiders in ecological webs. - Cambridge University Press.

Yuan T., Maun M. A. and Hopkins W. G. 1993. Effects of sand accretion on photosynthesis, leaf-water potential and morphology of 2 dune grasses. - Functional Ecology 7: 676-682.

Zar J. H. 1996. Biostatistical analysis. - Prentice-Hall.

Zarghami S., Allahyari H., Bagheri M. R. and Saboori A. 2010. Effect of nitrogen fertilization on life table parameters and population growth of *Brevicoryne brassicae*. - Bulletin of Insectology 63: 39-43.

Zoon F. C., Troelstra S. R. and Maas P. W. T. 1993. Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophae rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. - Fundamental and Applied Nematology 16: 247-258.

11 Supplementary Information

Table S3.1 Chemical analyses of soils from the highly dynamic *Ammophila* foredunes (left panel) and the more stabilised main dunes with pure Ammophiletum (right panel) at Braunton Burrows. Results are expressed per g dry weight of soil (source: Table 5 in Willis et al. 1959a. Journal of Ecology 47: 1-24).

pH		9.05	9.06
Organic carbon	mg	0.52	0.19
Total nitrogen	mg	0.18	0.11
Calcium	mg	70.4	69.5
Magnesium	µg	2270	990
Sodium	µg	528	14
Potassium	µg	50	6
Carbonate	mg	119.6	115.4
Phosphate-P	µg	109	110
Chloride	µg	845	14

Text S3.2 Differences in soil characteristics between different treatment soils, according to abiotic and biotic soil component and soil region of origin

Mineral nitrogen content (both NO_3-N and NH_4-N) was extracted in a 1:5 soil to 1 M KCl solution according to ISO 14256-2 and measured with a Foss Fiastar 5000 continuous flow analyser. Soil moisture content (expressed on fresh weight) was determined as the weight loss at 105 °C. The pH was measured in a 1M KCl solution according to ISO 10390. Inorganic C (IC) was measured with a Skalar Primacs SLC TOC-analyser. This result was converted to $CaCO_3$ content. Organic matter content in the soil was estimated according to EN 13039. Plant available P was determined by the method of Olsen et al. (1954).

The amount of NO_3-N significantly differed between soils with a different inoculum (Fig. S3.6, Table S3.3). Fully sterile soils contained less NO_3-N than soils with either of both non-sterile inocula (Table S3.4). This indicates that soil fauna were able to increase the amount of soil nitrate. If anything, plants performed worse on inoculated soils. Therefore this increase in soil nitrate did not benefit plants in our study. The NH_4-N content or moisture content did not significantly differ between any of the soil treatments. There was a

significant interactive effect of abiotic soil and region on the percentage $CaCO_3$ as well as on the percentage of organic matter and the pH (Fig. S3.7a, Table S3.3). Both at Le Perroquet and Ter Yde, soils from dynamic dunes had a higher $CaCO_3$ content than soils from stabilised dunes, with the highest content found in dynamic dune soil from Ter Yde. The pH of the soil mixtures significantly differed according to an interaction between region and abiotic soil component (Fig. S3.7b, Table S3.3). Both at Le Perroquet and Westhoek, soils with a sterile component from dynamic dunes had a higher pH than stabilised dune soils (Table S3.4). Percentage of organic matter per dry matter significantly differed between different combinations of abiotic soil component and region (Fig. S3.7b, Table S3.3). Soils from Westhoek and Le Perroquet had a higher organic matter content when the abiotic soil component originated from stabilised dunes compared to soils with a sterile part from dynamic dunes, while the opposite was true for soils from Ter Yde (Table S3.4). The amount of plant available phosphorus differed according to a significant region x soil x inoculum interaction (Fig. S3.8, Table S3.3, Table S3.4).

Table S3.3 Results of the final permutational ANOVA models of the different soil parameters, obtained after a stepwise backward selection procedure. R: region where soil was collected: Westhoek, Ter Yde or Le Perroquet. S: sterile, abiotic component of the soil: dynamic dunes or stabilised dunes. I: non-sterile, biotic soil inoculum: none, dynamic dunes or stabilised dunes. No significant effects were detected for the percentage moisture and NH_4-N (mg kg^{-1}) of the soils.

Source	df	SS	MS	pseudo-F	p	unique perms
NO3-N (mg/kg)						
I	2, 51	2406.8	1203.4	5.7658	0.0057	73019
pH-KCl						
R	2, 48	0.46815	0.23407	5.175	0.0077	95318
S	1, 48	1.1557	1.1557	25.552	0.00001	90408
RxS	2, 48	0.41926	0.20963	4.6346	0.0137	95441
% organic matter/dry matter						
R	2, 48	0.1908	0.0954	20.786	0.00001	95511
S	1, 48	0.15254	0.15254	33.233	0.00001	90738
RxS	2, 48	0.45234	0.22617	49.276	0.00001	95355
% CaCO3						
R	2, 48	9.9176	4.9588	11.846	0.0001	95378
S	1, 48	16.192	16.192	38.681	0.00001	90511
RxS	2, 48	8.9113	4.4557	10.644	0.0002	95384
Plant available P (mg/kg)						
R	2, 36	40.193	20.097	161.67	0.00001	95425
S	1, 36	1.8297	1.8297	14.719	0.0004	90681
I	2, 36	0.0630	0.0315	0.25337	0.7804	95479
RxS	2, 36	15.603	7.8017	62.761	0.00001	95507
RxI	4, 36	0.13354	0.0334	0.26857	0.8978	95608
SxI	2, 36	1.4319	0.71594	5.7594	0.0062	95582
RxSxI	4, 36	1.9355	0.48387	3.8926	0.0092	95387

Table S3.4 Results of pairwise comparisons among levels of factors that significantly affected the soil parameters in the final permutational ANOVA models. Comparisons were made by means of permutational t-tests. When the number of unique permutations was lower than 100, Monte Carlo sampling was used to obtain reliable p-values. Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde. Soil - D: sterile soil component of dynamic dune, S: sterile soil component of stabilised dune. Inoculum - /: no inoculum, d: dynamic dune biota, s: stabilised dune biota.

Groups	df	pseudo-t	p	unique perms	p (MC)
NO3-N					
/ - d	34	2.9549	0.0058	1471	-
/ - s	34	2.8711	0.0075	1644	-
d - s	34	0.27735	0.7827	1370	-
pH-KCl					
PE,D - PE,S	16	2.7727	0.0081	19	0.0139
PE,D - TY,D	16	3.7811	0.0042	13	0.0017
PE,D - TY,S	16	3.8933	0.0018	15	0.0014
PE,D - WE,D	16	1.6557	0.1482	12	0.1185
PE,D - WE,S	16	13.252	0.00003	28	0.00001
PE,S - TY,D	16	1.0396	0.3942	17	0.3136
PE,S - TY,S	16	0.57932	0.6552	18	0.571
PE,S - WE,D	16	1.2689	0.2609	23	0.2237
PE,S - WE,S	16	2.3117	0.0448	21	0.0341
TY,D - TY,S	16	0.73521	0.5707	10	0.4747
TY,D - WE,D	16	0.55989	0.674	14	0.5833
TY,D - WE,S	16	7.3472	0.00005	20	0.00001
TY,S - WE,D	16	1.0398	0.3642	17	0.317
TY,S - WE,S	16	5.2507	0.0004	19	0.00006
WE,D - WE,S	16	5.0328	0.0009	25	0.0001
% organic matter/dry matter					
PE,D - PE,S	16	2.4771	0.0291	36	0.0246
PE,D - TY,D	16	0.76964	0.5137	37	0.4547
PE,D - TY,S	16	1.9505	0.0746	57	0.0687
PE,D - WE,D	16	1.7165	0.1137	50	0.1063
PE,D - WE,S	16	12.646	0.00004	117	0.00001
PE,S - TY,D	16	0.98263	0.4352	31	0.342
PE,S - TY,S	16	3.5711	0.0051	67	0.0027
PE,S - WE,D	16	3.4101	0.0048	61	0.0033
PE,S - WE,S	16	10.271	0.00001	98	0.00001
TY,D - TY,S	16	2.2209	0.0462	63	0.0411
TY,D - WE,D	16	2.0246	0.0676	61	0.0603
TY,D - WE,S	16	8.5979	0.00004	104	0.00001
TY,S - WE,D	16	0.25245	0.8207	60	0.804
TY,S - WE,S	16	10.624	0.00004	146	0.00001
WE,D - WE,S	16	10.75	0.00004	141	0.00001

Table S3.4: continued.

% $CaCO_3$					
PE,D - PE,S	16	2.9779	0.0082	394	0.0089
PE,D - TY,D	16	3.2156	0.007	426	0.0051
PE,D - TY,S	16	2.8422	0.0114	446	0.0115
PE,D - WE,D	16	2.8033	0.0138	370	0.0128
PE,D - WE,S	16	3.6072	0.0025	359	0.0025
PE,S - TY,D	16	6.4534	0.0002	591	0.00002
PE,S - TY,S	16	0.1867	0.8578	313	0.8553
PE,S - WE,D	16	0.28751	0.7834	273	0.7764
PE,S - WE,S	16	0.50859	0.6173	249	0.6168
TY,D - TY,S	16	6.0123	0.0003	643	0.00004
TY,D - WE,D	16	6.3542	0.0003	596	0.00002
TY,D - WE,S	16	7.2643	0.0001	596	0.00002
TY,S - WE,D	16	0.43833	0.6692	332	0.6685
TY,S - WE,S	16	0.2284	0.8302	294	0.8211
WE,D - WE,S	16	0.8483	0.4114	259	0.4106
Plant available P					
PE,D,/ - PE,D,d	4	1.1543	0.4013	10	0.313
PE,D,/ - PE,D,s	4	0.43329	0.7016	8	0.6884
PE,D,/ - PE,S,/	4	5.7183	0.1017	10	0.0046
PE,D,/ - PE,S,d	4	8.5208	0.1001	10	0.0011
PE,D,/ - PE,S,s	4	5.0964	0.1009	10	0.0066
PE,D,/ - TY,D,/	4	8.9317	0.0995	10	0.001
PE,D,/ - TY,D,d	4	7.4831	0.0998	10	0.0018
PE,D,/ - TY,D,s	4	20.575	0.0997	10	0.00001
PE,D,/ - TY,S,/	4	5.5385	0.1005	10	0.005
PE,D,/ - TY,S,d	4	12.823	0.0995	10	0.0001
PE,D,/ - TY,S,s	4	18.033	0.1006	9	0.0001
PE,D,/ - WE,D,/	4	4.222	0.0995	10	0.0133
PE,D,/ - WE,D,d	4	7.6896	0.0988	10	0.0016
PE,D,/ - WE,D,s	4	6.5463	0.1004	10	0.0028
PE,D,/ - WE,S,/	4	20.019	0.0994	10	0.00002
PE,D,/ - WE,S,d	4	21.5	0.1012	10	0.00003
PE,D,/ - WE,S,s	4	6.8147	0.0997	10	0.0026
PE,D,d - PE,D,s	4	1.6207	0.303	10	0.1809
PE,D,d - PE,S,/	4	4.0989	0.1	10	0.0147
PE,D,d - PE,S,d	4	4.6323	0.0999	10	0.0097
PE,D,d - PE,S,s	4	3.1384	0.1007	10	0.0345
PE,D,d - TY,D,/	4	7.8804	0.1002	10	0.0015
PE,D,d - TY,D,d	4	6.9547	0.0997	10	0.0023
PE,D,d - TY,D,s	4	17.144	0.0988	9	0.00007
PE,D,d - TY,S,/	4	5.0503	0.1004	10	0.0073
PE,D,d - TY,S,d	4	9.8311	0.1	10	0.0008
PE,D,d - TY,S,s	4	11.152	0.1002	10	0.0004
PE,D,d - WE,D,/	4	3.222	0.0998	10	0.032

Table S3.4: continued.

PE,D,d - WE,D,d	4	4.7752	0.0988	10	0.009
PE,D,d - WE,D,s	4	5.0685	0.0997	10	0.0067
PE,D,d - WE,S,/	4	14.747	0.098	10	0.0001
PE,D,d - WE,S,d	4	16.243	0.101	10	0.00007
PE,D,d - WE,S,s	4	6.114	0.1002	10	0.0035
PE,D,s - PE,S,/	4	6.5889	0.1002	9	0.0026
PE,D,s - PE,S,d	4	14.473	0.0998	10	0.00007
PE,D,s - PE,S,s	4	6.3661	0.0996	10	0.0031
PE,D,s - TY,D,/	4	9.3939	0.1003	10	0.0008
PE,D,s - TY,D,d	4	7.6696	0.0998	10	0.0017
PE,D,s - TY,D,s	4	22.96	0.0997	10	0.00002
PE,D,s - TY,S,/	4	5.7001	0.1013	10	0.0047
PE,D,s - TY,S,d	4	15.009	0.1024	10	0.0001
PE,D,s - TY,S,s	4	30.86	0.1006	10	0.00001
PE,D,s - WE,D,/	4	4.6247	0.1005	10	0.0096
PE,D,s - WE,D,d	4	10.346	0.0986	9	0.0005
PE,D,s - WE,D,s	4	7.2912	0.0994	10	0.0018
PE,D,s - WE,S,/	4	25.222	0.0989	10	0.00001
PE,D,s - WE,S,d	4	26.419	0.0994	10	0.00003
PE,D,s - WE,S,s	4	7.0774	0.0985	10	0.002
PE,S,/ - PE,S,d	4	1.226	0.4032	10	0.2865
PE,S,/ - PE,S,s	4	1.5132	0.2003	9	0.2031
PE,S,/ - TY,D,/	4	5.0391	0.0999	10	0.007
PE,S,/ - TY,D,d	4	5.1858	0.0997	10	0.0064
PE,S,/ - TY,D,s	4	12.197	0.1013	10	0.0003
PE,S,/ - TY,S,/	4	3.3069	0.099	10	0.0286
PE,S,/ - TY,S,d	4	4.8674	0.1007	10	0.008
PE,S,/ - TY,S,s	4	4.3681	0.0996	10	0.0115
PE,S,/ - WE,D,/	4	6.54E-02	1	10	0.9514
PE,S,/ - WE,D,d	4	0.5167	0.7017	7	0.6303
PE,S,/ - WE,D,s	4	1.2863	0.4001	10	0.2668
PE,S,/ - WE,S,/	4	8.619	0.1001	10	0.0009
PE,S,/ - WE,S,d	4	10.155	0.0996	10	0.0003
PE,S,/ - WE,S,s	4	3.8775	0.1002	10	0.0184
PE,S,d - PE,S,s	4	0.79152	0.4997	10	0.4722
PE,S,d - TY,D,/	4	6.3816	0.1009	10	0.0032
PE,S,d - TY,D,d	4	5.8504	0.0987	10	0.0047
PE,S,d - TY,D,S	4	17.866	0.101	10	0.00005
PE,S,d - TY,S,/	4	3.8794	0.0999	10	0.0178
PE,S,d - TY,S,d	4	8.7825	0.0995	10	0.0009
PE,S,d - TY,S,s	4	17.5	0.1017	9	0.00006
PE,S,d - WE,D,/	4	0.91818	0.5016	10	0.411
PE,S,d - WE,D,d	4	1.16	0.4018	10	0.3106
PE,S,d - WE,D,s	4	2.6997	0.1002	10	0.0515
PE,S,d - WE,S,/	4	17.408	0.1007	10	0.00004

Table S3.4: continued.

PE,S,d - WE,S,d	4	19.143	0.0994	9	0.0001
PE,S,d - WE,S,s	4	4.7234	0.1009	10	0.0092
PE,S,s - TY,D,/	4	6.2915	0.1011	10	0.0034
PE,S,s - TY,D,d	4	5.9036	0.1005	10	0.0044
PE,S,s - TY,D,s	4	15.425	0.1005	10	0.0001
PE,S,s - TY,S,/	4	3.9799	0.099	10	0.0167
PE,S,s - TY,S,d	4	7.435	0.1005	9	0.0018
PE,S,s - TY,S,s	4	8.3593	0.1005	10	0.0012
PE,S,s - WE,D,/	4	1.204	0.2993	10	0.2961
PE,S,s - WE,D,d	4	1.4297	0.3016	10	0.2262
PE,S,s - WE,D,s	4	2.7866	0.1009	9	0.049
PE,S,s - WE,S,/	4	12.64	0.1002	10	0.0003
PE,S,s - WE,S,d	4	14.315	0.1004	10	0.0001
PE,S,s - WE,S,s	4	4.7846	0.0992	10	0.0091
TY,D,/ - TY,D,d	4	1.7416	0.3	10	0.1555
TY,D,/ - TY,D,s	4	3.2975	0.0988	10	0.0293
TY,D,/ - TY,S,/	4	4.60E-02	1	10	0.9661
TY,D,/ - TY,S,d	4	2.0798	0.1998	10	0.1071
TY,D,/ - TY,S,s	4	3.2745	0.1018	10	0.031
TY,D,/ - WE,D,/	4	4.4095	0.0987	10	0.012
TY,D,/ - WE,D,d	4	5.8271	0.0991	10	0.0046
TY,D,/ - WE,D,s	4	3.9109	0.1006	10	0.0166
TY,D,/ - WE,S,/	4	0.2193	0.9008	9	0.8344
TY,D,/ - WE,S,d	4	0.85723	0.5993	9	0.4389
TY,D,/ - WE,S,s	4	0.18853	0.8987	10	0.8615
TY,D,d - TY,D,s	4	0.21067	0.8994	10	0.846
TY,D,d - TY,S,/	4	1.3955	0.1992	10	0.2359
TY,D,d - TY,S,d	4	3.2725	0.1	9	0.0303
TY,D,d - TY,S,s	4	3.9848	0.1004	10	0.0161
TY,D,d - WE,D,/	4	4.8749	0.1003	10	0.0083
TY,D,d - WE,D,d	4	5.5646	0.0988	10	0.0051
TY,D,d - WE,D,s	4	4.484	0.0999	10	0.0111
TY,D,d - WE,S,/	4	2.1249	0.201	10	0.1028
TY,D,d - WE,S,d	4	1.4516	0.4	10	0.2211
TY,D,d - WE,S,s	4	1.7522	0.2021	10	0.153
TY,D,s - TY,S,/	4	2.0834	0.2	10	0.1057
TY,D,s - TY,S,d	4	8.4474	0.1015	10	0.001
TY,D,s - TY,S,s	4	12.455	0.0994	10	0.0002
TY,D,s - WE,D,/	4	9.6031	0.1013	10	0.0007
TY,D,s - WE,D,d	4	15.702	0.0999	10	0.00006
TY,D,s - WE,D,s	4	9.8912	0.0995	9	0.0006
TY,D,s - WE,S,/	4	6.198	0.1005	10	0.0035
TY,D,s - WE,S,d	4	4.3262	0.1009	10	0.0122
TY,D,s - WE,S,s	4	2.9027	0.101	9	0.044
TY,S,/ - TY,S,d	4	1.3656	0.2986	10	0.2458

Table S3.4: continued.

TY,S,/ - TY,S,s	4	2.0137	0.0988	9	0.1138
TY,S,/ - WE,D,/	4	3.0965	0.1013	10	0.0363
TY,S,/ - WE,D,d	4	3.6165	0.0986	10	0.0226
TY,S,/ - WE,D,s	4	2.64	0.0998	10	0.0573
TY,S,/ - WE,S,/	4	0.18757	0.7988	10	0.8582
TY,S,/ - WE,S,d	4	0.47853	0.699	10	0.6584
TY,S,/ - WE,S,s	4	0.18785	0.8003	10	0.8595
TY,S,d - TY,S,s	4	2.1638	0.1989	7	0.0969
TY,S,d - WE,D,/	4	3.6518	0.0991	10	0.0219
TY,S,d - WE,D,d	4	7.0278	0.1001	10	0.0021
TY,S,d - WE,D,s	4	3.0058	0.1006	10	0.0398
TY,S,d - WE,S,/	4	3.661	0.0997	10	0.0211
TY,S,d - WE,S,d	4	5.5224	0.1002	10	0.0055
TY,S,d - WE,S,s	4	1.4437	0.3012	10	0.2223
TY,S,s - WE,D,/	4	2.9045	0.1016	10	0.0443
TY,S,s - WE,D,d	4	8.8403	0.0996	10	0.0011
TY,S,s - WE,D,s	4	2.0697	0.2016	10	0.1091
TY,S,s - WE,S,/	4	8.7563	0.1005	10	0.0009
TY,S,s - WE,S,d	4	11.133	0.1003	10	0.0003
TY,S,s - WE,S,s	4	2.3056	0.1001	10	0.0825
WE,D,/ - WE,D,d	4	0.44573	0.8007	9	0.6801
WE,D,/ - WE,D,s	4	0.98803	0.5015	10	0.3814
WE,D,/ - WE,S,/	4	6.2138	0.0999	10	0.0036
WE,D,/ - WE,S,d	4	7.4182	0.0992	9	0.0019
WE,D,/ - WE,S,s	4	3.5229	0.1007	10	0.0244
WE,D,d - WE,D,s	4	1.9911	0.1994	10	0.1161
WE,D,d - WE,S,/	4	13.279	0.0991	10	0.0002
WE,D,d - WE,S,d	4	15.105	0.0994	10	0.0001
WE,D,d - WE,S,s	4	4.3525	0.0989	10	0.0119
WE,D,s - WE,S,/	4	6.0988	0.0993	10	0.0038
WE,D,s - WE,S,d	4	7.5288	0.1006	10	0.0018
WE,D,s - WE,S,s	4	3.0119	0.0996	9	0.0389
WE,S,/ - WE,S,d	4	2.3413	0.2026	9	0.0799
WE,S,/ - WE,S,s	4	5.78E-02	0.9009	10	0.9563
WE,S,d - WE,S,s	4	0.90939	0.5009	9	0.4149

Table S3.5 Results of pairwise comparisons among levels of factors that significantly affected plant and aphid characteristics in the final permutational ANOVA models. Comparisons were made by means of permutational t-tests. When the number of unique permutations was lower than 100, Monte Carlo sampling was used to obtain reliable p-values. Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde. Inoculum - /:no inoculum, d: dynamic dune biota, s: stabilised dune biota.

Groups	df	pseudo-t	p	unique perms	p (MC)
Aphids					
Maximum # aphids					
PE, TY	108	3.5856	0.0007	618	-
PE, WE	106	1.525	0.132	272	-
TY, WE	108	2.2681	0.0254	582	-
Generation time					
PE, TY	108	0.08866	0.9654	68	0.9295
PE, WE	106	1.941	0.0476	55	0.0554
TY, WE	108	1.8968	0.0601	111	0.0601
Growth constant					
PE, TY	108	1.6854	0.0959	14490	-
PE, WE	106	0.82767	0.4157	9342	-
TY, WE	108	2.6324	0.0091	14962	-
Plants					
Total dry mass					
PE,/ - PE,d	21	0.79632	0.5023	2574	-
PE,/ - PE,s	25	0.44043	0.7	2980	-
PE,/ - TY,/	23	1.3687	0.1859	4307	-
PE,/ - TY,d	23	2.5635	0.0071	3076	-
PE,/ - TY,s	21	0.71371	0.5073	1711	-
PE,/ - WE,/	19	1.9281	0.0529	1494	-
PE,/ - WE,d	20	1.3289	0.2052	3001	-
PE,/ - WE,s	18	0.94134	0.3898	2794	-
PE,d - PE,s	24	0.65177	0.5231	1087	-
PE,d - TY,/	22	2.3737	0.0266	3678	-
PE,d - TY,d	22	3.0053	0.0067	2046	-
PE,d - TY,s	20	4.77E-02	0.963	1205	-
PE,d - WE,/	18	2.2495	0.0394	1828	-
PE,d - WE,d	19	0.99887	0.3279	2028	-
PE,d - WE,s	17	0.50239	0.6276	1839	-
PE,s - TY,/	26	2.2135	0.0364	3832	-
PE,s - TY,d	26	3.4582	0.0023	1393	-
PE,s - TY,s	24	0.53076	0.601	1436	-
PE,s - WE,/	22	2.6083	0.018	2313	-
PE,s - WE,d	23	1.5068	0.1424	2469	-
PE,s - WE,s	21	0.9657	0.3444	2285	-

Table S3.4: continued.

TY,/ - TY,d	24	4.1195	0.0005	2341	-
TY,/ - TY,s	22	2.1235	0.0457	4118	-
TY,/ - WE,/	20	3.2457	0.0048	2135	-
TY,/ - WE,d	21	2.7163	0.0141	4030	-
TY,/ - WE,s	19	2.1923	0.0415	3773	-
TY,d - TY,s	22	1.9901	0.0578	1391	-
TY,d - WE,/	20	0.43026	0.6681	1758	-
TY,d - WE,d	21	1.3275	0.1961	2170	-
TY,d - WE,s	19	1.5695	0.1341	2017	-
TY,s - WE,/	18	1.4171	0.1739	2479	-
TY,s - WE,d	19	0.68315	0.5002	2689	-
TY,s - WE,s	17	0.32852	0.7442	2499	-
WE,/ - WE,d	17	0.82191	0.4174	1947	-
WE,/ - WE,s	15	1.0662	0.3137	1775	-
WE,d - WE,s	16	0.31065	0.7597	2104	-
Root/total dry mass					
/, d	66	2.3204	0.0082	9196	-
/, s	66	0.11853	0.9325	5781	-
d, s	66	2.5971	0.0008	7697	-

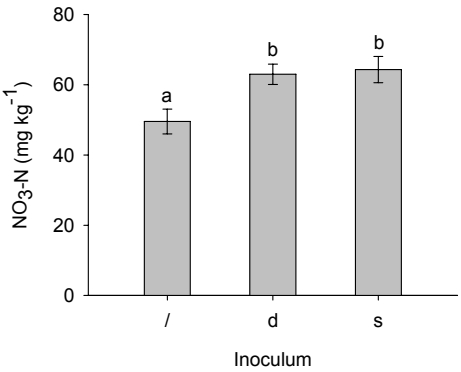


Figure S3.6 Differences in NO_3-N content of treatment soils with different non-sterile inocula (mean + SE). Significant pairwise differences are indicated by different letters above the bars ($p < 0.05$). Inoculum - /: no inoculum, d: dynamic dune biota, s: stabilised dune biota.

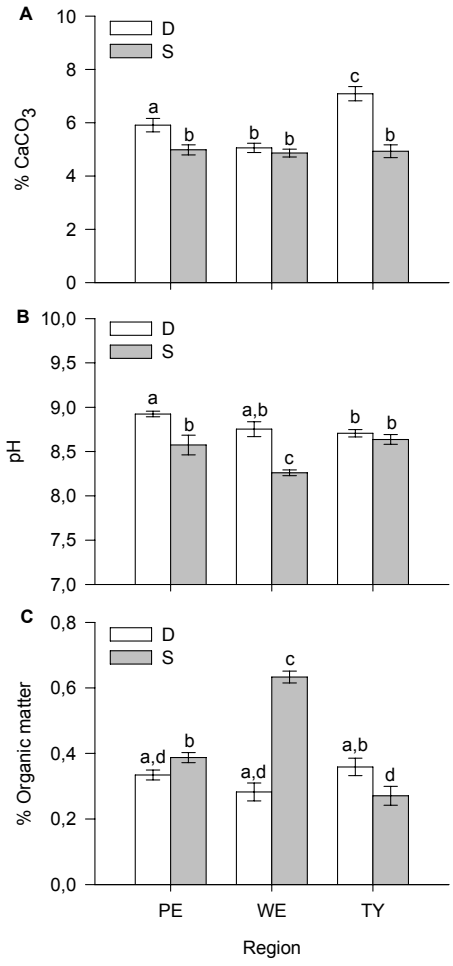


Figure S3.7 Differences in soil parameters of treatment soils according to region and abiotic soil component (mean + SE). A) Percentage $CaCO_3$. B) pH-KCl. C) Percentage organic matter per dry matter. Significant pairwise differences are indicated by different letters above the bars ($p < 0.05$). Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde. Soil - D: sterile soil component of dynamic dune, S: sterile soil component of stabilised dune.

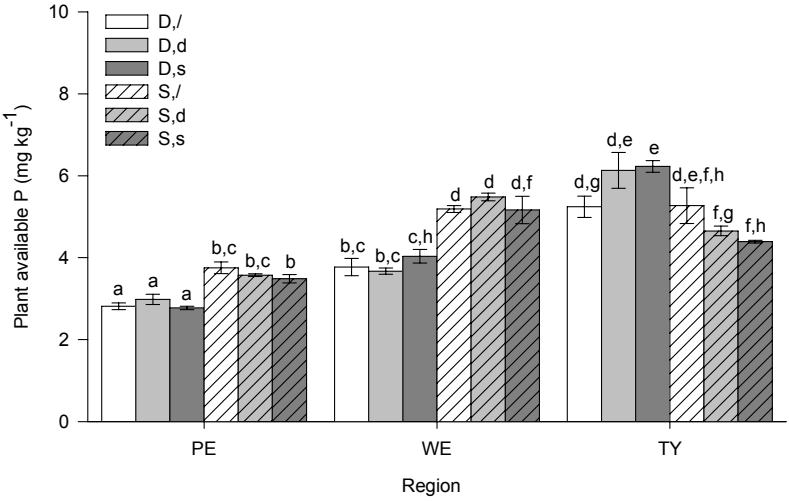


Figure S3.8 Differences in plant available P of treatment soils according to region, abiotic soil component and non-sterile soil inoculum (mean + SE). Significant pairwise differences are indicated by different letters above the bars ($p < 0.05$). Region - PE: Le Perroquet, WE: Westhoek, TY: Ter Yde. Soil - D: sterile soil component of dynamic dune, S: sterile soil component of stabilised dune. Inoculum - /: no inoculum, d: dynamic dune biota, s: stabilised dune biota.

Text S5.1 Details of plant growing protocol and differences in characteristics of *Ammophila arenaria* plants between populations at planting and at harvest.

Seeds were germinated at a light regime of 8/16 hours dark/light in plastic pots filled with a 3 cm layer of sterilised dune sand (1 hour at 120 °C and 1 atm), saturated with demineralised water. Plastic lids of the pots were perforated to allow of enough ventilation. Moisture level was reset to near saturation 3 times a week. Seedlings were transplanted to open plastic pots of 1 l filled with sterile dune sand after two weeks and watered daily for two weeks and 3 times per week from then onwards. After three weeks, the light regime was changed to 10/14 hours dark/light. From 6 weeks onwards tap water was given twice a week. Plants were transplanted to large plastic pots with defaunated sand (one night at 200 °C) as they reached a height of approximately 1 metre, and 100 ml of half strength Hoagland solution was given at six occasions spread in time. Two weeks before being installed in the field, 200 ml of fertiliser (Compo NPK 16-9-20, 2 g l^{-1} tap water) was administered.

For each plant, prior to installation in the field, the total number of tillers was recorded and for 5 randomly chosen tillers the number of leaves per tiller and the length of the longest leaf were determined. At the end of the experiment, one day before harvest, these measurements were repeated.

To assess whether plants from different populations differed in characteristics at planting and at harvest, one-way ANOVAs were performed with population as a factor. Tested variables were: the number of tillers, the average number of leaves per tiller and the average leaf height, each of them at planting and at harvest, the root fresh weight at harvest, the shoot fresh weight at harvest, the vitality of the root system at harvest and the relative water content of the shoot at harvest. Only fresh weight of roots could be determined, since fresh roots were needed for subsequent staining. Shoot fresh weight is therefore used in all analyses for comparability with root fresh weight. Since the correlation between fresh and dry shoot weight was almost perfect (Pearson correlation coefficient = 0.98995), this does not affect any conclusions. The average number of leaves per tiller at harvest, the average length of leaves at harvest and the relative water content of the

shoot at harvest were squared and the root fresh weight at harvest was square root transformed to meet the assumption of normality of residuals. For the number of tillers at planting and the squared length of leaves at harvest, the assumption of homogeneity of variances was not met, and Welch's variance-weighted ANOVA was used.

Plant populations significantly differed in their number of tillers, both at planting ($F_{5,24.67} = 9.96$, $p < 0.0001$) and at harvest ($F_{5,54} = 5.85$, $p = 0.0002$), with the order of populations remaining similar over the course of the experiment (Fig. S5.4a). The number of leaves per tiller also significantly differed between plant populations at planting ($F_{5,54} = 6.89$, $p< 0.0001$), but no longer at harvest (Fig. S5.4b). The tillers of all plants lost some leaves over the duration of the experiment, with higher losses for plants with a lot of leaves. The length of the leaves differed among populations (Fig. S5.4c) at planting ($F_{5,54} = 17.04$, $p< 0.0001$) and at harvest ($F_{5,24.87} = 3.01$, $p = 0.029$). Differences were more pronounced at planting, and all plants grew shorter leaves in the field than in the laboratory. If all three plant features are considered together, it can be concluded that all plants decreased in size, but that the order of plant sizes across plant populations did not change over the course of the experiment. Both root fresh weight ($F_{5,54} = 9.57$, $p< 0.0001$) and shoot fresh weight ($F_{5,54} = 4.24$, $p = 0.0025$) significantly differed at harvest (Fig. S5.5a). Root vitality, the proportion of 30 randomly chosen 1 cm fragments with more than half of the cortex intact, did not significantly differ between populations ($F_{5,54} = 2.27$, $p = 0.060$). Relative water content of the shoots differed significantly between plant populations ($F_{5,54} = 5.11$, $p = 0.0007$; Fig. S5.5b). These results point out the importance of plant characteristics as covariates in the analyses of above- and belowground invertebrate occurrence in response to plant population.

Text S5.2 Relationship between invertebrate species, individual *Ammophila arenaria* plants and plant populations as visualised by Nonmetric Multidimensional Scaling.

To visualise the relationship between species, plants and plant populations, we performed a Nonmetric Multidimensional Scaling (NMS) ordination. This ordination arranges data points, here plants, in a three-dimensional space according to their distance in multidimensional space. Plants with a similar invertebrate species composition are thus plotted closer to each other in this new space. Species are depicted by vectors that originate in the centroid of the point cloud and whose projections on each axis represent the correlation with the gradient represented by that axis. Plants from different populations are represented by different symbols. Ordination was based on the Bray-Curtis distance matrix of plants and obtained by 50 randomisations with a stability criterion of 0.001. To give equal weight to all species in the ordination, individual occurrences were standardised by species totals.

The NMS resulted in a minimum stress of 0.188 for a three-dimensional solution, and a value of 0.262 for a two-dimensional solution. A stress value smaller than 0.2 is generally considered to be a useful representation, while values between 0.2 and 0.3 should be treated with caution. We therefore chose to depict the three-dimensional solution from the angle of view that maximises the length of the species vectors, thus showing the maximum amount of variation in the point cloud. This ordination plot (Fig. S5.6) visualises the relationship between plant genotype and invertebrate occurrences. Although the plants from same populations do not form distinct clusters in multispecies space, there is a shift from more distant populations to the local populations throughout the diagram. The first division in the cluster analysis, i.e. between above- and belowground invertebrates is again reflected in the negative correlation of the three nematode species (Md, P and H) and a positive correlation of all the aboveground invertebrates with the Belgian populations. Note that within the aboveground invertebrates, the species that formed clusters in the hierarchical clustering analysis are again highly correlated, for example the species *R. padi*, *L. psammae* and *P. plagiatum*. So although this ordination is based on a Bray-Curtis distance between individual plants, it matches the clustering of species, based on correlations across genotypes, quite well.

Figure S5.3 Location of the six sampled populations of *Ammophila arenaria* in Europe. B: Belgium, N: the Netherlands, P: Portugal, S: Spain.

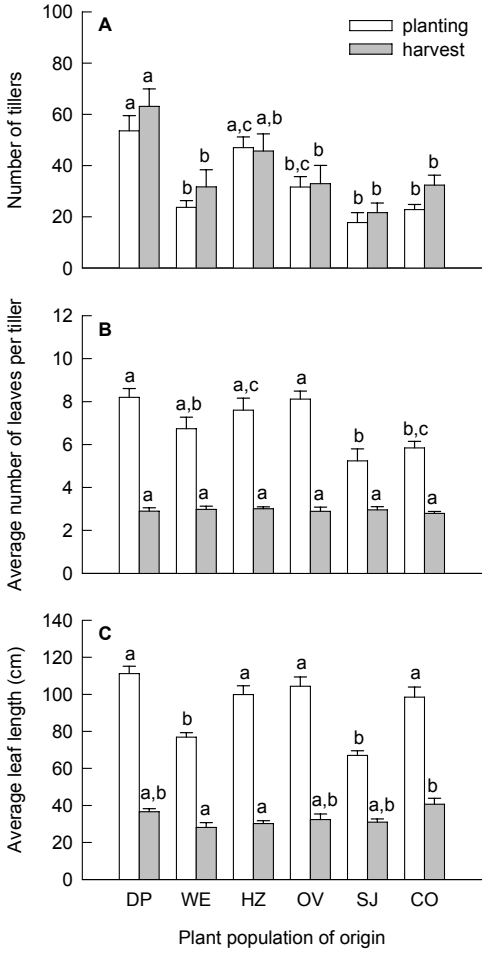


Figure S5.4 Characteristics of *Ammophila arenaria* plants, at planting and at harvest, from populations of different geographical origin (mean + SE). In order of ascending distance to the local population: DP: De Panne - Belgium, WE: Westende - Belgium, HZ: Het Zwin - Belgium, OV: Oostvoorne - Netherlands, SJ: São Jacinto - Portugal, CO: Comporta - Portugal. A: number of tillers. B: number of leaves per tiller, averaged over 5 randomly chosen tillers. C: Length of the longest leaf, averaged over 5 randomly chosen tillers. Significant differences between means are indicated by different letters above the bars following Tukey's hsd post hoc test ($p < 0.05$).

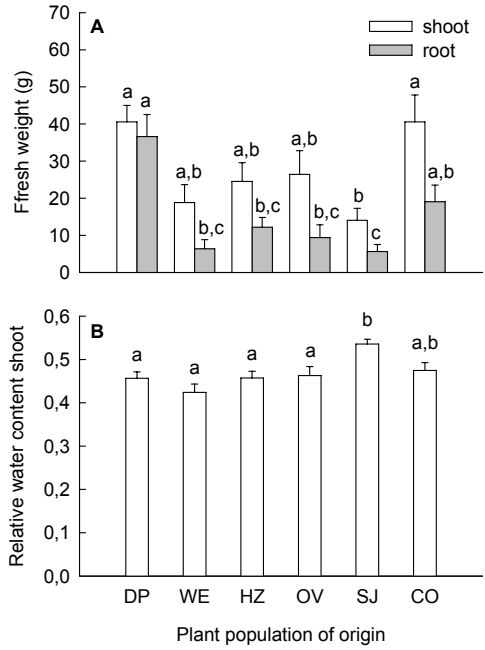


Figure S5.5: A) Root and shoot fresh weight and B) relative water content of shoots at harvest of *Ammophila arenaria* plants from populations of different geographical origin (mean + SE). In order of ascending distance to the local population: DP: De Panne - Belgium, WE: Westende - Belgium, HZ: Het Zwin - Belgium, OV: Oostvoorne - Netherlands, SJ: São Jacinto - Portugal, CO: Comporta - Portugal. Significant differences between means are indicated by different letters above the bars following Tukey's hsd post hoc test ($p < 0.05$).

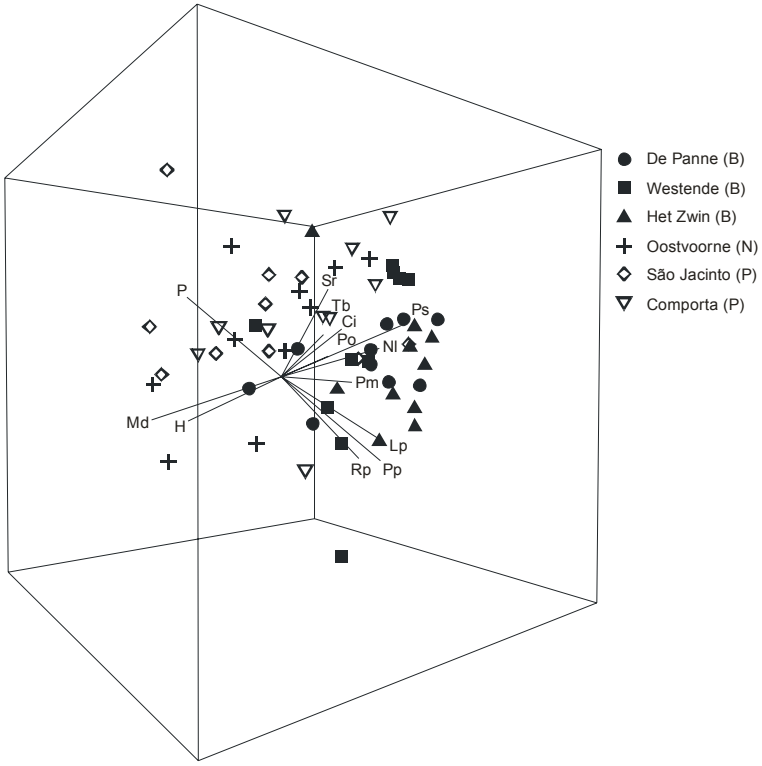


Figure S5.6: NMS ordination of individual *Ammophila arenaria* plants according to their invertebrate fauna. Three-dimensional NMS joint plot. Each point represents an individual *Ammophila arenaria* tussock. Different plant populations are indicated by different symbols. B: Belgium, N: Netherlands, P: Portugal. Invertebrate occurrences are represented by lines that radiate from the centroid of the point cloud. The projection of a line on an axis represents the r^2 value between that variable and the axis. Ci: *Candidula intersecta*, H: *Heterodera* sp., Lp: *Laingia psammae*, Md: *Meloidogyne duytsi*, Nl: *Neophilaenus lineatus*, P: *Pratylenchus* sp., Pm: *Psammotettix maritimus*, Po: *Phalangium opilio*, Pp: *Philopedon plagiatum*, Ps: *Porcellio scaber*, Rp: *Rhopalosiphum padi*, Sr: *Schizaphis rufula*, Tb: *Theba pisana*.

Text S6.1: Details of AFLP analyses

Of each population, ten seedlings were harvested when they were three weeks old. Fresh seedlings were immediately frozen in liquid nitrogen. In the lab, they were freeze-dried for 48h and homogenized with a mill (Retsch MM 200) to fine powder. Five mg of dried leaf material was used for DNA extraction using the QuickPick™ plant DNA kit (Bio-Nobile) on a MagRo™ 8-M robotic workstation (Bio-Nobile). DNA quality was checked on 1.5% agarose gels. Concentration and purity were determined using a ND-1000 spectrophotometer (NanoDrop, Thermo Scientific). Hundred ng of DNA was used for AFLP analysis according to Vos et al. (1995). Restriction and ligation were done in a single step. Amplification of fragments was performed in two steps using the primer combinations EcoRI+A/MseI+C and EcoRI+C/MseI+G for preamplification and EcoRI+ATC/MseI+CAT, EcoRI+ATC-/MseI+CCA, EcoRI+ACA/MseI+CAC, EcoRI+ACA/MseI+CCG, EcoRI+CCA/MseI+GTT, EcoRI+CCA/MseI+GGA, EcoRI+CAT/MseI+GAT EcoRI+CAT/MseI+GCT for selective amplification. Fragment separation and detection took place on a Nen IR^2 DNA analyzer (Licor) using 24 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50 to 700 bp) were included for sizing of the fragments. Control samples were included to check for reproducibility. Only clear, intense polymorphic bands between 75 bp and 500 bp were scored. Scoring was done using the SAGAmx software (Licor). We scored the presence or absence of every marker in each individual as 1 or 0 (present or absent) to form a binary data matrix.

Table S6.2. Pairwise population Φ_{PT} values (below diagonal) and probability values based on 999 permutations (above diagonal).

	DP	WE	HZ	OV	SJ	CO
DP		0.001	0.019	0.125	0.001	0.001
WE	0.323		0.002	0.001	0.001	0.001
HZ	0.138	0.229		0.011	0.001	0.001
OV	0.057	0.385	0.173		0.001	0.001
SJ	0.788	0.787	0.783	0.787		0.002
CO	0.838	0.835	0.834	0.837	0.202	

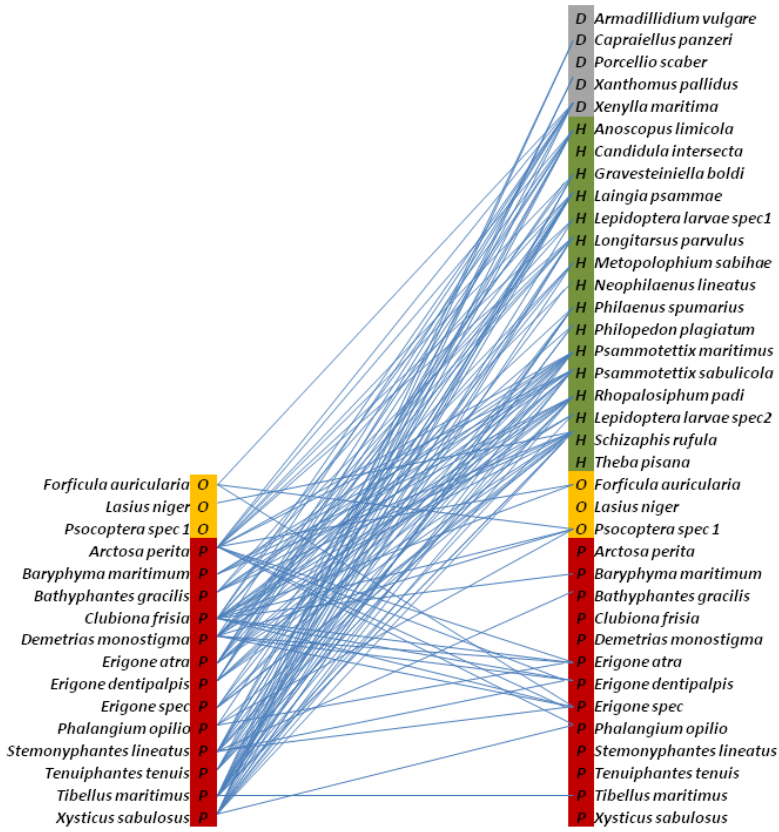


Figure S6.3. Graphical representation of the invertebrate food web associated with *Ammophila arenaria*, based on commonly occurring species. Links are based on predator-prey body size ratios. Note that this web represents all the commonly occurring predator and prey species of *A. arenaria*. Therefore, any actual food web is a subset of the web represented here. D: detritivore, H: herbivore, O: omnivore, P: predator.

Curriculum Vitae

Personal data

Last name:	Vandegehuchte
First names:	Martijn Lodewijk
Date of birth:	8 May 1984
Place of birth:	Ostend, Belgium
Nationality:	Belgian

Education

2002:	High school degree, specialisation: sciences-mathematics with 8 hours of mathematics per week. Onze Lieve Vrouwecollege, Ostend, Belgium.
2004:	Bachelor of Science degree in Biology, magna cum laude. Ghent University, Belgium.
2006:	Master of Science degree in Biology, magna cum laude. Ghent University, Belgium.
	Thesis: Effects of flooding dynamics, landscape configuration and habitat quality on the composition and functional characterisation of ground beetle communities of gravel banks along the Common Meuse (in Dutch).

Scientific career

2006-2010:	PhD student supported by a predoctoral fellowship of the Research Foundation – Flanders (FWO).

Academic experience

Supervision of students:

2007-2008: Bachelor of Science in Biology thesis: Roeland Cortois (with Dr. Eduardo de la Peña). The role of plant-parasitic nematodes in the *Ammophila* problem: results of a field study. Ghent University, Belgium.

2008-2009: Master of Science in Nematology thesis: Bart Vandenbossche (with Prof. Wim Bert and Dr. Eduardo de la Peña). Ecological linkages between above- and belowground herbivores: Insights from the interaction between plant-parasitic nematodes and aphids in the dune grass *Ammophila arenaria*. Ghent University, Belgium.

2009-2010: Master of Science in Biology thesis: Roeland Cortois (with Prof. Dries Bonte and Dr. Eduardo de la Peña). How do drought stress, root herbivory and mycorrhizal symbiosis affect aboveground invertebrate herbivores? Experimental evidence from *Ammophila arenaria* (Poaceae). Ghent University, Belgium.

Teaching:

Once every academic year during the following periods:

2006-2010: Practical for the course Biological Excursions: identification of ground beetles (Coleoptera: Carabidae). Second year of Bachelor in Biology.

2006-2010: Supervision of a research topic at the field week for the course Field Biological Research. Third year of Bachelor in Biology.

2007-2010: Guest lecture for the course Terrestrial Ecology: plant-insect interactions. Third year of Bachelor in Biology.

2007-2010: Practical for the course Biosphere Animals: identification of major arthropod taxa. First year of Bachelor in Geology.

Publications

Publications in international journals, included in S.C.I.:

Lambeets K., Vandegehuchte M. L., Maelfait J.-P. and Bonte D. 2008. Understanding the impact of flooding on trait-displacements and shifts in assemblage structure of predatory arthropods on river banks. - Journal of Animal Ecology 77: 1162–1174.

de la Peña E., Vandegehuchte M. L., Bonte D. and Moens M. 2008. Analysis of the specificity of three root-feeders towards grasses in coastal dunes. - Plant and Soil 310: 113-120.

Lambeets K., Vandegehuchte M. L., Maelfait J.-P. and Bonte D. 2009. Integrating environmental conditions and functional life-history traits for riparian arthropod conservation planning. - Biological Conservation 142: 625-637.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. - Oikos 119: 1011-1019.

Bonte D., de Roissart A., Vandegehuchte M. L., Ballhorn D. J., Van Leeuwen T. and de la Peña E. 2010. Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota. - PLoS ONE 5: e11174.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Aphids on *Ammophila arenaria* in Belgium: First reports, phenology and host range expansion. - Belgian Journal of Zoology 140: 77-79.

Vandegehuchte M.L., de la Peña E. and Bonte D. 2010. Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics.- PLoS ONE 5: e12937.

Vandegehuchte M.L., de la Peña E. and Bonte D. in press. Contrasting covariation of above- and belowground invertebrate species across plant genotypes. - Journal of Animal Ecology.

Bonte D., Breyne P., Brys R., de la Peña E., D'hondt B., Ghyselen C., Vandegehuchte M. L., and Hoffmann M. in press. Landscape dynamics determine the small-scale genetic structure of an endangered dune slack plant species. - Journal of Coastal Research.

Publications in preparation or submitted:

Vandegehuchte M. L., Cortois R., de la Peña E. and Bonte D. under review. Mycorrhizal fungi decrease *Ammophila arenaria* seedling growth and aphid population size. - Acta Oecologica .

Vandegehuchte M. L., de la Peña E., Breyne P. and Bonte D. under review. Non-local genotypes of a resident plant species reduce species richness of the invertebrate community and alter food web structure. - Ecology.

Wiersma E. R., Bonte D., Vandegehuchte M. L., Warui C. and Lens L. submitted. Spatio-temporal variation in the diversity of predatory arthropods in the tropics: orb weaving spiders in space and time. - Oikos.

de la Peña E., Vandegehuchte M. L., Bonte D. and Moens M. submitted. Nematodes surfing the waves: dispersal of root-feeding nematodes via sea swept rhizomes. - Functional Ecology.

Abstracts of scientific presentations:

Lambeets K., Vandegehuchte M. L. Bonte D. and Maelfait J.-P. 2006. The use of spider and carabid beetle assemblages for habitat evaluation and restoration management along a lowland gravel river (the Common Meuse, Belgium). 1st European Congress of Conservation Biology, 22-26 August, Eger, Hungary. Oral presentation.

Vandegehuchte M. L. 2007. Effects of dune stabilisation on life history, dispersal capacity and community structure of arthropods associated with *Ammophila arenaria*. International PhD course: 'Soil Ecology; Crossing the frontier between below- and above-ground', 5-9 February, Wageningen, the Netherlands. Oral presentation.

Vandegehuchte M. L., Maelfait J.-P. and Bonte D. 2007. Effects of dune stabilisation on life history, dispersal capacity and community structure of arthropods associated with *Ammophila arenaria*. Doctoraatssymposium Faculteit Wetenschappen UGent, 24 April, Ghent. Poster.

Vandegehuchte M. L., Bonte D. and Maelfait J.-P. 2007. Does dispersal limitation affect the occurrence of marram grass associated invertebrates? Insights from a field survey. 37th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), 10-14 September, Marburg, Germany. Oral presentation.

de la Peña E., Vandegehuchte M. L. and Bonte D. 2007. Specificity of plant-parasitic nematodes towards grasses in coastal dunes. 14th Benelux

Congress of Zoology, 1-2 November, Amsterdam, The Netherlands. Poster.

Vandegehuchte M. L., Bonte D., de la Peña E. and Maelfait J.-P. 2008. Importance of belowground plant features in explaining aboveground herbivore distribution patterns. Multitrophic Interactions Workshop, 6-7 March, Göttingen, Germany. Poster.

Vandegehuchte M. L., Bonte D., de la Peña E. and Maelfait J.-P. 2008. Importance of belowground plant features in explaining aboveground herbivore distribution patterns. Symposium of the Spanish Association of Terrestrial Ecology: 'The evolutionary ecology of plant-animal interactions: From genes to communities', 21-23 April, Palma de Mallorca, Illes Balears, Spain. Poster.

Bonte D., Bossuyt B., de la Peña E. and Vandegehuchte, M. L. 2008. How small herbivores induce wind dispersal. Symposium of the Spanish Association of Terrestrial Ecology: 'The evolutionary ecology of plant-animal interactions: From genes to communities', 21-23 April, Palma de Mallorca, Illes Balears, Spain. Poster.

Vandegehuchte M. L., de la Peña E., Maelfait J.-P. and Bonte D. 2008. Factors affecting the assembly of above- and belowground communities of marram vegetations. EURECO-GFOE 2008, a joint meeting of the European Ecological Federation (EEF) and the Ecological Society of Germany, Switzerland and Austria (GfÖ), 15-19 September, Leipzig, Germany. Oral presentation.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2009. Relative importance of biotic and abiotic soil components in their effect on plant growth and insect herbivore population dynamics. Doctoraatssymposium Faculteit Wetenschappen UGent, 28 April, Ghent, Belgium. Poster.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2009. Plant genotype differentially affects colonization by generalist and specialist insect herbivores. 94th Ecological Society of America (ESA) Annual Meeting, 2-7 August, Albuquerque, New Mexico, USA. Oral presentation.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2009. Interactions between above- and belowground invertebrate fauna associated with *Ammophila arenaria* in dynamic coastal dunes. VLIZ Young Scientists' Day, 27 November, Ostend, Belgium. Oral presentation and poster.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Plant genotype differentially affects above- and belowground invertebrates. Netherlands Annual Ecology Meeting 2010, 9-10 February, Lunteren, the Netherlands. Oral presentation.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Contrasting covariation of above- and belowground invertebrates across plant

genotypes. 95th Ecological Society of America (ESA) Annual Meeting, 1-6 August, Pittsburgh, Pennsylvania, USA. Oral presentation.

Vandenbossche B., Vandegehuchte M. L., Bert W., Bonte D. and de la Peña E. 2010. Plant genotype affects abundance of plant-parasitic nematodes and aphids on the dune grass *Ammophila arenaria*. 30th meeting of the European Society of Nematology, 19-23 September, Wien, Austria. Poster.

Vandegehuchte M. L., de la Peña E. and Bonte D. 2010. Contrasting covariation of above- and belowground invertebrate species across plant genotypes. Symposium 'Entomology in Belgium 2010', 3 December, Brussels, Belgium. Oral presentation.

ISBN 978-90-9025921-5